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Textbook of the Autoimmune Diseases,
Edited by R. G. Lahita, N. Chiorazzi, and W. H. Reeves,
Lippincott Williams & Wilkins, Philadelphia 2000.

CHAPTER 15

Autoimmunity of the Gastrointestinal Tract

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MORPHOLOGY AND TERMINOLOGY OF CHRONIC GASTRITIS

The importance of classification of chronic gastritis is the causal relation of some cases to pernicious anemia, peptic ulcer, and adenocarcinoma. By identifying combinations of morphologic features, topographic distribution of the abnormalities and the association with other variables, it is possible to define different subtypes (1). Chronic atrophic gastritis is recognized macroscopically by the loss of gastric mucosal folds and thinning of the gastric mucosa layer. Depending on the localization of the lesions, the disease can be classified into two types. Type A autoimmune gastritis is restricted to the fundus and body of the stomach, whereas type B gastritis involves the antrum and comprises also the fundus and the body. Type A gastritis is associated with pernicious anemia, autoantibodies to gastric parietal cells, and to intrinsic factor, achlorhydria, low serum pepsinogen levels, and elevated serum gastrin concentrations. The latter results from hyperplasia of gastrin-producing cells. Type B gastritis is generally associated with the *Helicobacter pylori* infection accompanied by low serum gastrin concentrations because of destruction of the gastrin-producing cells. One of the cardinal histologic features of all subtypes is inflammation, which is primarily chronic (i.e., composed of lymphocytes and plasma cells). However, inflammation may have a variable acute component of neutrophils.

PERNICIOUS ANEMIA

Pernicious anemia, the most common cause of vitamin B_{12} deficiency, is of diverse pathophysiologic origins (2). The term *pernicious anemia* applies solely to the conditions associated with chronic atrophic gastritis. Pernicious anemia, originally described by Thomas Addison in 1849, was linked to the stomach by Austin Flint in 1860 (3). The discovery of a serum inhibitor of intrinsic factors, later identified as an autoantibody to intrinsic factor (4), and of autoantibodies to parietal cells (5) led to the deduction that immunologic disturbances underlay the gastritis that causes pernicious anemia. The risk of gastric carcinoids seems to be high in this patient group compared with a normal population, but the lesions are mostly relatively benign tumors (6).

Pathologic Findings

Gastric biopsy specimens from patients with pernicious anemia show a mononuclear cellular infiltrate in the submucosa extending into the lamina propria between the gastric glands. The infiltrate includes plasma cells, T cells, and a population of non-T cells. The infiltrating plasma cells contain autoantibodies to the antigen of parietal cells and to intrinsic factor (7,8).

Clinical Manifestations

The median age at diagnosis of pernicious anemia is 60 years because of the late onset and slow progression of the disease (9). There is a slight prevalence of women. The usual presentation is linked with symptoms of anemia. The vitamin B_{12} deficiency results in several abnormalities of the digestive tract, such as smooth and red tongue caused by atrophic glossitis, diarrhea, and megaloblastosis of the epithelial cells. Vitamin B_{12} deficiency may cause peripheral neuropathy and lesions in the posterior and lateral columns of the spinal cord and cerebrum. These lesions progress from demyelination to axonal degeneration and neuronal death. The most frequent manifestations of peripheral neuropathy include paresthesias and numbness. The manifestations of lesions in the spinal cord are, for example, loss of position sense and sensory

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ataxia spasticity. Cerebral symptoms range from mild personality defects and memory loss to psychosis.

Immunologic Manifestations

The most important feature of pernicious anemia is the presence of circulating autoantibodies directed against parietal cells in more than 90% patients. The autoantigen was found as a microsomal component in sections of human gastric mucosa by means of immunofluorescence and complement fixation techniques. It was further localized to the microvilli of the parietal cells. Later, the gastric H^+/K^+-adenosine triphosphatase (ATPase) acid pump was demonstrated to constitute the major autoantigen of the parietal cell (10–13). The ATPases are a highly conserved family of proteins responsible for the ATP-dependent transport of ions across the membranes of mammalian cells that includes Na^+/K^+-ATPase and Ca^{2+}-ATPase. These enzymes have a highly conserved 100-kd catalytic subunit (α) that undergoes phosphorylation during reaction cycles and have a 60- to 90-kd glycoprotein subunit (β). The α and β subunits are required for the ATPase activity. One major parietal cell autoantigen recognized by the human antibodies is the α subunit of the gastric H^+/K^+-ATPase. A second major parietal cell antigen targeted by the human autoantibodies is a 60- to 90-kd glycoprotein (14). The antibodies inhibited the enzymatic activity of H^+/K^+-ATPase, and their binding site was reported to be located on the cytoplasmic side of H^+/K^+-ATPase.

The α and β subunits of human H^+/K^+-ATPase were cloned, and their nucleotide sequences have been determined. This made it possible to apply recombinant DNA techniques for generation of recombinant peptides and to characterize the epitopes on human H^+/K^+-ATPase. The major epitope is located in the NH_2-terminal part of the α subunit between residues 360 and 525 on the cytosolic side of the secretory membrane (15). Gastric H^+/K^+-ATPase appears to be the only parietal cell antigen recognized by circulating parietal cell autoantibodies. Immunoblotting and immunoprecipitation experiments revealed reactivity solely within the two subunits of this ATPase.

Identification of gastric H^+/K^+-ATPase as the autoantigen raises a question about the role of the ATPase in the immunopathogenesis of the gastric lesions. Development of an animal model of autoimmune gastritis, organ-specific autoimmune disease, represents a new experimental approach that could bring more insights into the pathogenesis of this disorder. Murine autoimmune gastritis can be generated in susceptible mice strains after neonatal thymectomy, treatment of newborn mice with cyclosporine, or immunization with gastric H^+/K^+-ATPase (16). However, transgenic expression of the β subunit of the ATPase prevents experimental induced gastritis. These findings suggest that autoimmune gastritis occurs only when pathogenic T cells are transferred to immunocompromised mice and that the pathogenic T cells have been rendered tolerant after overexpression of β subunit.

The antiparietal cell antibody commonly found in pernicious anemia often occurs in subjects with gastritis who do not have pernicious anemia. Antibody to intrinsic factor (IF) rarely occurs in atrophic gastritis that is not accompanied by vitamin B_{12} malabsorption, and it has been suggested that such cases represent a predisease state (17,18). For that reason, it is considered to be more specific marker for pernicious anemia than antiparietal cell antibody. IF is a protein that is produced by gastric parietal cells and binds to and facilitates the absorption of vitamin B_{12}. Antibodies to IF are found in serum of about 55% of patients with pernicious anemia, many of whom have such antibodies in gastric secretion. The role of IF antibodies in the development of pernicious anemia is not clear. Although they prevent IF-mediated vitamin B_{12} absorption and are almost exclusively confined to patients with pernicious anemia, nearly half such patients do not have IF antibodies in serum or gastric juice, and there are no apparent differences between those with and those without IF antibodies. Southern blot analysis of genomic DNA from patients with congenital pernicious anemia (lacking IF) revealed normal restriction fragment patterns, suggesting that a sizable gene deletion was not responsible for the IF deficiency (19).

Mechanism of Vitamin B_{12} Malabsorption

Gastric parietal cells produce IF, a 60-kd glycoprotein that can bind dietary vitamin B_{12}. The complex of vitamin B_{12} and IF is transferred to the distal ileum, where it is absorbed after binding to the specific receptors on the luminal membranes. IF deficiency leads to the malabsorption of vitamin B_{12}. At least two mechanisms are responsible for the absence of IF. The progressive destruction and loss of parietal cells impairs its secretion. Autoantibodies present in the gastric juice can block the vitamin B_{12} binding site of the IF and prevent complex formation. Because the vitamin B_{12} is required for DNA replication, the vitamin B_{12} deficiency affects most frequently organs exhibiting rapid cell turnover such as the gastrointestinal tract and the bone marrow.

Diagnostic Approach

Examination of the peripheral blood reveals macrocytosis with hypersegmented polymorphonuclear leukocytes, leukopenia, anemia, thrombocytopenia, or pancytopenia. Determination of B_{12} and folate concentrations in serum is the first important diagnostic test followed by a Schilling test. A low B_{12} concentration and normal folate level are characteristic for pernicious anemia. An elevated concentration of fasting serum gastrin and low pepsinogen level are associated with pernicious anemia (20). If the diagnosis is not definite proof, examination of a bone marrow aspirate is necessary. Megaloblastic hemopoiesis documented by the presence of megaloblasts and large myeloid precursors is characteristic of the disease (21).

Therapy

The standard therapy is regular monthly intramuscular injections of at least 100 μg of vitamin B_{12} to restore its deficiency. For elderly patients with gastric atrophy, taking tablets containing 25 μg to 1 mg of vitamin B_{12} daily has been recommended to prevent B_{12} deficiency. This treatment remedies the anemia and may correct the neurologic complications.

ACHLORHYDRIA

Achlorhydria or diffuse antral gastritis in its pure form is not primarily atrophic but is characterized by a mixed acute and chronic mucosal inflammation. It tends not to extend beyond the antrum and is strongly associated with the presence of *H. pylori*. The gram-negative gastric pathogen causes lifelong infections leading to gastritis and to gastric and duodenal ulceration and mucosa-associated lymphoid tissue (MALT) lymphoma (1,22–24).

Pathophysiologic Characteristics of *Helicobacter pylori*–Infected Patients

Patients with *H. pylori*–induced antigastric antibodies differ in a number of histopathologic parameters from those without autoimmune manifestations. They develop antigastric autoantibodies of various specificity, have increased numbers of T cells infiltrating the corpus glandular epithelium, have increased numbers of polymorphonuclear leukocytes invading the corpus, have an elevated epithelial apoptosis rate in the corpus, and have increased occurrence and severity of corpus atrophy (25–29). Moreover, the patients have increased blood gastrin levels, accompanied by decreased acid secretion and a lowered pepsinogen I : II ratio.

Autoantibodies to Gastric–Mucosal Antigens

Infection with *H. pylori* induces autoantibodies reactive with gastric parietal cell canaliculi. Parietal cells secrete gastric acid by a mechanism involving the proton pump (i.e., the gastric H^+/K^+-ATP-ase), which is localized in the apical secretory canaliculi (30). It has been shown that *H. pylori* lipopolysaccharide (LPS) expresses Lewis x and y blood group antigens, which are also present on gastric epithelial cells (31). It seemed plausible that an initial event for the epitope specificity of *H. pylori*–induced autoantibodies is molecular mimicry (32,33). It has been reported that animals immunized or infected with *H. pylori* develop antibodies directed against *H. pylori* LPS, parietal H^+/K^+-ATPase (32), and Lewis x/y antigens (34). However, infected patients develop antibodies reacting with *H. pylori* LPS and native gastric H^+/K^+-ATPase but not with Lewis x/y antigens. Binding to gastric H^+/K^+-ATPase or to canaliculi was not abolished by preadsorption with *H. pylori* cells. These data indicate that the infected patients develop autoantibodies driven by gastric H^+/K^+-ATPase, not because of molecular mimicry (34). Moreover, several sera recognize a 45-kd glycoprotein, probably IF, implicating epitope spreading to other autoantigens.

Putative Pathogenesis of *Helicobacter pylori*–Induced Autoimmunity

Infection with *H. pylori* results in gastric mucosal influx of $CD4^+$T cells and in a type 1 helper T-cell (T_H1) response. In the gastric tissue, increased levels of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-12 (IL-12), and corresponding mRNAs were detected (35,36). *H. pylori* infection may induce IL-12, which is responsible for selection of IFN-γ–producing T_H1 cells. IFN-γ results in aberrant expression of major histocompatibility complex (MHC) class II molecules on gastric epithelium instead of on professional antigen-presenting cells. Increased MHC class II molecules stimulate enhanced presentation of autoantigens such as gastric H^+/K^+-ATPase, leading to activation of the immune cascade. After increased autoantigen presentation, autoreactive T cells become activated, inducing autoantibodies and resulting in destruction of glands, such as by execution of FAS-FASL–mediated apoptosis.

Induction of autoimmunity after infection is not unique feature of *H. pylori* but a common characteristic of many pathogens. For example, *Campylobacter jejuni* induces Guillain-Barré syndrome.

Diagnostic Approach

Type B gastritis (37–40) is characterized by a regenatory epithelium, depletion of mucus, lymphoid follicles, intestinal metaplasia, and focal atrophy. Rod-like microorganisms corresponding to *H. pylori* are observed on the surface.

Therapy

Treatment of *H. pylori* infection consists of proton pump inhibitors (PPIs) and antibiotics, most notably clarithromycin and metronidazole. PPI therapy is widely used, but long-term treatment with these drugs in subjects who are *H. pylori* positive may increase their risk of developing gastric cancer.

AUTOIMMUNE HEPATITIS

Classification

Autoimmune hepatitis (AIH) (41,42) is an inflammatory liver disease characterized histologically by a dense mononuclear cell infiltrate in the portal tract and serologically by the presence of non–organ- and liver-specific autoantibodies and increased levels of immunoglobulin G (Fig. 15.1).

AIH is associated with circulating antibodies to cellular components. On the basis of different autoantibodies specificities, AIH is subdivided into three groups (43–47). Type I AIH is associated with antibodies to nuclear (ANA) and/or smooth muscle (SMA) autoantigens, whereas type II AIH is

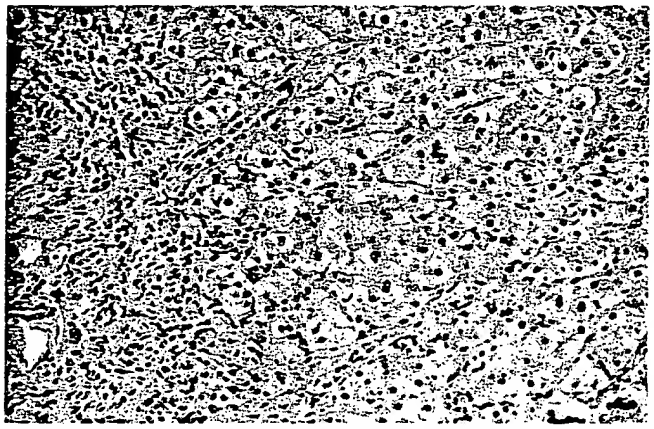

FIG. 15.1. Inflammatory reaction in autoimmune hepatitis (AIH).

characterized by antibodies to liver-kidney microsomal (LKM1) antigen. In addition, a substantial number of patients with AIH produce antibodies to a cytosolic soluble liver antigen (SLA) alone or in combination with ANA and/or SMA. Whether the latter patients represent a distinct AIH entity is controversial. However, this antibody had proved to be an extremely useful diagnostic marker and may help in identifying patients who are seronegative for other autoantibodies.

Antinuclear Antibodies and Autoimmune Hepatitis

Heterogeneity of Antinuclear Antibodies

Patients with AIH spontaneously produce ANAs (45,48) that are directed against nucleic acids and various nuclear proteins of their own cells. Although the pathogenic role of such antibodies was not demonstrated, they represent specific disease markers and are useful biologic tools.

ANAs were initially recognized by the LE cell test and now can be easily detected by indirect immunofluorescence microscopy using tissue preparations as a substrate. Commercially available Hep-2 cells are most frequently used for routine screening for ANAs. The application of uniformly prepared cultured cell substrates or tissue sections from manufacturers offers the possibility to standardize the testing for ANAs. In the case of a positive reaction, the titer of ANAs after the sequential serum dilution can be determined, and the staining pattern can be clearly analyzed.

The frequency of ANAs in AIH is about 70% of cases at the time of presentation according to the earlier (42) and later studies (48). However, these data should be carefully considered regarding the results of a study of International Union of Immunological Societies (IUIS) Standardization Committee (49). According to this report, the ranges of frequencies of ANA positivity on Hep-2 cells for normal subjects strongly depends on serum dilution (49). The respective frequencies at serum dilution of 1 : 40, 1 : 80, 1 : 160, and 1 : 320 were 32%, 13%, 5%, and 3%, respectively, demonstrating the critical balance between sensitivity and specificity of the tests at different dilutions. The interpretation of the ANA frequency in AIH should be made in light of the previously described study findings to avoid overestimation.

The immunofluorescent patterns exhibited by ANA-positive human sera vary from homogenous, speckled, centromere, rimlike, and nuclear dots to nucleolar and may indicate the subcellular localization of target antigens (Fig. 15.2). In the case of a characteristic staining pattern such as rimlike or nucleolar, it can be expected that the autoantibodies are directed against constituents of the corresponding subnuclear structure as nuclear envelope or nucleoli. Homogenous or speckled nuclear staining is less informative, because these types of reactivity are common for several nuclear antigens. Moreover, most autoimmune sera are heterogeneous and contain different antibodies, resulting in overlapping staining patterns.

Indirect immunofluorescence is an easy and rapid assay for the detection of autoantibodies in the clinical routine, but it is not suitable for identification of responsive antigens. For this purpose, two other methods are employed: immunoblotting (often called Western blotting) and immunoprecipitation. Although both methods make use of electrophoresis and allow determination of the size and optionally charge of autoantigens, they are not equivalent but rather complementary. During immunoblotting assay, antigens immobilized on membrane are at least partially denatured, whereas immunoprecipitation tests are performed with native antigens. Other methods such as enzyme-linked immunosorbent assay (ELISA) or immunodots are also available. Although they are sensitive, they show greater variability than other assays (50). The quality of the antigen used as its purity or lack of proteolytic degradation cannot be directly verified. Because of these important limitations, both methods seem to be more suitable for quantitative estimations than for qualitative determinations.

The presence of circulating ANAs is one of the diagnostic criteria for AIH. ANAs are a heterogeneous group of antibodies (51,52) that are also detectable in other liver disorders. Four major groups of ANAs have been described in patients with AIH: anti-DNA, antihistones, antinucleoprotein particles, and antinonhistone proteins. For a better understanding of the properties and physiologic function of the autoantibodies, it is advisable to characterize their corresponding antigens.

Anti-DNA Antibodies

Most cellular DNA occurs in the nucleus in form of complexes with various structural and regulatory proteins. Naked DNA is susceptible to degradation by endogenous nucleases or mechanical injury, and the formation of complexes with proteins protects DNA from them. Native DNA occurs mostly as a double-stranded helix and adopts different higher-order conformations, of which the B structure is the most common. Complex formation with nuclear proteins may alter the conformation of DNA. There are many DNA-binding proteins. Among these structural proteins are some such as the histones that bind nucleic acids independent of

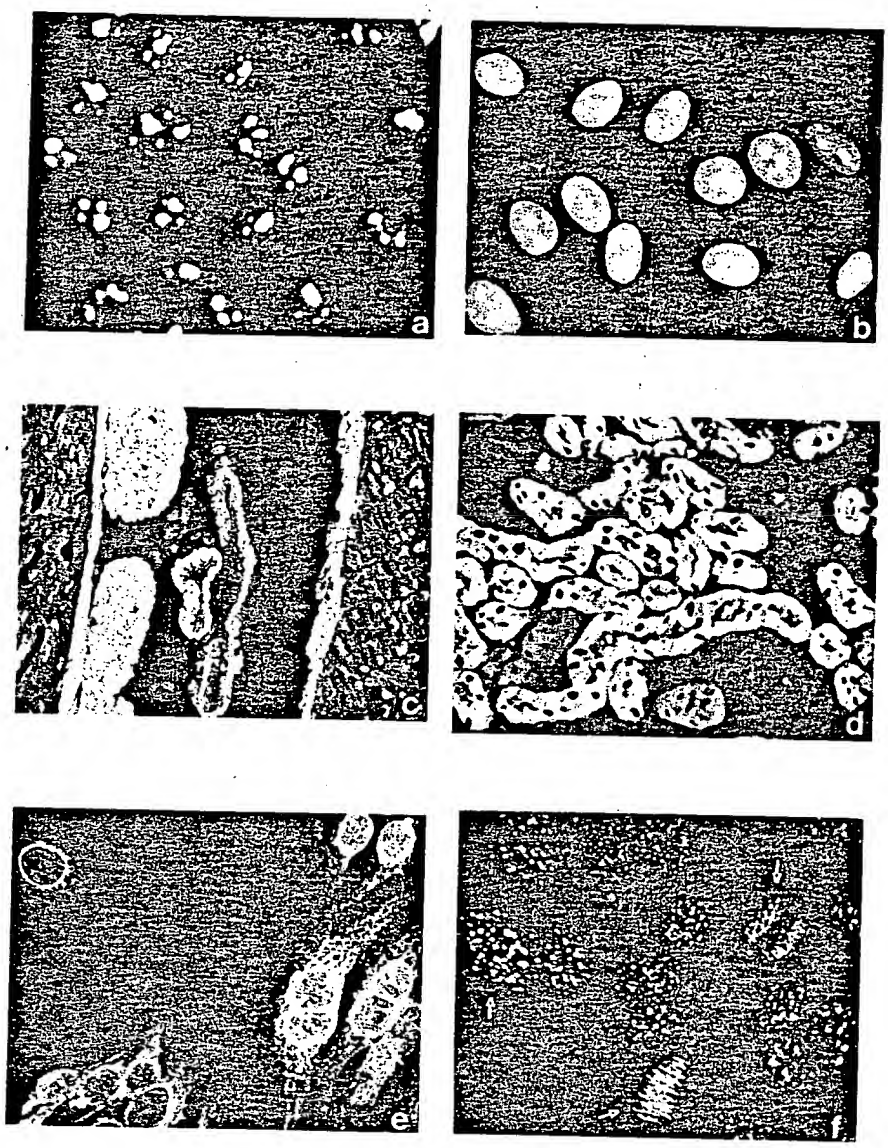

FIG. 15.2. Immunofluorescence patterns exhibited by autoimmune hepatitis sera. For detection of autoantibodies, HEp-2 cells **(A, E, F)**, HeLa cells **(B)**, or rodent tissue preparations were used. **A:** Nucleolar staining pattern. **B:** Homogenous plus nucleolar pattern. **C:** Pattern shown by antismooth muscle antibodies (SMAs). **D:** liver-kidney microsomal (LKM) antigen. **E:** Rimlike pattern. **F:** Centromere pattern, with cells in different stages of the cell cycle *(arrows)*.

quence, and there are proteins that bind to specific sences of DNA.

The classic B conformation is considered to be weakly mmunogenic or nonimmunogenic. Immunogenic nucleic id forms include single-stranded DNA and RNA, RNA-NA hybrids (duplexes and triplexes) and Z-DNA. Modid DNA, such as methylated or carcinogen-substituted NA, and DNA-protein complexes are thought to be pot immunogens (51,52).

There are two main types of anti-DNA antibodies (53–55). tibodies targeting single-stranded DNA (ssDNA) recoge pyrimidine and purine bases in denatured DNA and are reactive with double-stranded DNA (dsDNA). The lack of reactivity with dsDNA is not surprising, because the bases are not accessible in native DNA. A second type of antibodies representing those reactive with dsDNA recognizes primarily the sugar-phosphate backbone and therefore is able to bind dsDNA or ssDNA.

Anti-DNA antibodies, found primarily in persons with systemic lupus erythematosus (SLE) (53–55) and implicated in the pathogenesis of the disease, were also observed in patients with AIH. Several studies have determined the frequency of anti-dsDNA antibodies in AIH (56,57).

Studies in the 1970s, based on liquid or solid phase immunoassays and commercial sources of antigen, ascertained a high frequency of positivity for anti-dsDNA in AIH pa-

tients, but positive results were also obtained for those with other liver disorders. Because the specificity of these assays depends strongly on the quality of the antigen used, it is impossible to exclude the possibility that even negligible contamination with ssDNA could lead to overestimation because of nonspecific reactivity. When more specific immunoassays for anti-dsDNA antibodies, such as Farr-type radioimmunoassays, membrane filtration, or *Crithidia luciliae* immunofluorescence, were used alone or in combination (58–60), the positivity rate was about 10% for AIH cases. In a later study (61), the frequency of anti-DNA antibodies in AIH determined by ELISA was about 50% (56 of 99 patients). Moreover, anti-DNA antibodies were detected in persons with chronic hepatitis B. The frequency and titers of anti-DNA antibodies of the IgG class in patients with AIH and chronic hepatitis B were similar. Mortality and response to therapy were not related to the presence or absence of these autoantibodies.

Anti-dsDNA antibodies occur seldom in AIH. Earlier assays and ELISA results tended to overestimate the frequency of positive results. Because the patients with or without antibodies could not be distinguished by clinical, histologic, and biochemical criteria, it has been suggested that the presence of anti-dsDNA antibodies in AIH is a nonspecific manifestation of inflammation.

Antihistone Antibodies

Histones are abundant, highly conserved nuclear proteins (62). Because of the high content of arginine and lysine residues, they are positively charged and bind to DNA in chromatin in a sequence-independent mode. Histone proteins are involved in compacting DNA and formation of primary and higher-order chromatin structure. Five main classes of histones—H1, H2A, H2B, H3, and H4—occur in the nucleus in a highly organized nucleosomal structure (63). Each nucleosomal particle, representing the smallest chromatin unit, consists of about 200 base pairs of DNA wrapped twice around a histone core, which contains two of each of the H2A, H2B, H4, and H4 histones in an octameric configuration. The H1 histones are associated with linker DNA at sites where DNA enters and exits core particles and connects one nucleosome to the next. Moreover, because of its cooperative binding, histone H1 is involved in formation of higher-order chromatin structure.

The primary structure of molecules may vary within each of the five classes of histone proteins. H1 is most heterogeneous of the histones, encompassing multiple H1 subtypes. The expression of distinct H1 subtypes and their relative concentrations differs in tissue- and species-specific manner and varies during differentiation and throughout development (64,65). All H1 subtypes possess three structurally distinct domains. A central globular domain consisting of 75 amino acids is the most conserved and is flanked by randomly coiled amino- and carboxyl-terminal parts. The carboxyl-terminal segment, rich in lysine, alanine, and proline, is highly basic. The heterogeneity of H1 histone proteins may be of functional importance in that the several H1 variants differentially regulate formation of the higher-order chromatin structure (65). The remaining histones occur also as subtypes. The variability of their primary structure has been determined. The major antigenic determinants in histones are known to occur in the variable regions of the amino and carboxyl termini (65–67). Because the regions are exposed on the surface of nucleosomes and are accessible to antibodies, the native chromatin structures may act as immunogens.

Autoantibodies to all classes of histone proteins are frequently found in autoimmune diseases and seem to be a characteristic feature of idiopathic and drug-induced SLE (66–69). With immunofluorescence, human sera positive for histones exhibit a homogenous nuclear staining pattern (Fig. 15.2B) correlating with the chromatin distribution. Antihistone antibodies in the free or DNA-complexed form have been detected in AIH patients' sera (70–72). The incidence of antihistone antibodies ranges from 25% to 35% (70–72). A detailed analysis using individual histones revealed the reactivity of AIH sera with all histones, but H2B was most prevalent. In one report, antihistone-positive cases were accompanied by active disease and associated with sicca syndrome (72). The available data indicate that antihistone antibodies are not a specific feature of AIH and are instead randomly generated in the course of inflammatory processes in the liver.

Antibodies to Nonhistone Proteins

Centromere Proteins. Antibodies to centromere proteins exhibit (ACAs) an anticentromere staining pattern by immunofluorescence (73–75). The centromere plays a major role in segregation of eukaryotic chromosomes in mitosis by serving as the site for chromatid attachment and kinetochore assembly (76). A characteristic feature of the anticentromere antibodies (ACAs) is differential staining pattern of the interphase and mitotic cells that helps distinguish them from other autoantibodies.

The antibodies recognize at least three well-defined centromere proteins—CENP-A (17 kd), CENP-B (80 kd), and CENP-C (140 kd)—as shown by immunoblotting with human nuclear proteins or by ELISA and using purified recombinant proteins (75,77,78). These centromere antigens are DNA-binding proteins; a sequence-specific DNA activity has been found only for CENP-B (79). CENP-B, located in the central domain of the centromere, possess a DNA-binding domain in the NH_2 terminus and dimerization domain in the COOH terminus (80). CENP-C is a highly basic protein located in the inner kinetochore plate, playing an important role in the assembly of centromere (75,81). CENP-A protein shows a high degree of sequence identity with histone H3 and shares with it a similar organization (75,82–84).

ACAs occur frequently in scleroderma (70% to 80%) and are characteristic of the CREST syndrome (*c*alcinosis, *R*aynaud's phenomenon, *e*sophageal hypomotility, *s*clerdactyly, *t*elangiectasias). The frequency of ACA estimated by

immunofluorescence, ELISA, or both assays was about 40% in cases of AIH. However, in our experience, the ACA determinations based solely on immunofluorescence (Fig. 15.2F) are not sufficiently substantiated by ELISA, and because of extreme variation, we were not able to reproduce the results (i.e., high percentage of anit–ACA-antibodies). This discrepancy can be explained by the overestimation if evaluation was based solely on immunofluorescence or ELISA. In our experience, the ACA determinations based solely on immunofluorescence pattern are not sufficiently substantiated and commercially available ELISA tests gave extremely varying results.

Antilamin Antibodies. Antibodies directed against nuclear lamins give a characteristic peripheral nuclear staining pattern by immunofluorescence, which is designated as rimlike (Fig. 15.2E). This type of reactivity indicates that antibodies recognize components of the nuclear envelope (85–87), but it does not detail the reactive antigens. The nuclear envelope, a supramolecular structure of interphase nucleus in eukaryotic cells, separates the nucleus from the cytoplasm (85–87). The most important function of the nuclear envelope is to give support for chromatin organized in the loops and to regulate exchange of macromolecules between the nucleus and cytoplasm.

The nuclear envelope consists of three morphologically and functionally distinct components: outer and inner nuclear membranes, the pore complexes, and the nuclear lamina. The outer nuclear membrane is connected with the rough endoplasmic reticulum. The outer and inner nuclear membranes are joined at multiple sites, where the nuclear pore complexes are anchored. At the nucleoplasmic side of the inner membrane, the nuclear lamina is attached.

The nuclear lamina, a proteinaceous meshwork, is composed of a relatively small number of proteins, which have been called lamins (85). Depending on the cell type and differentiation state, this structure contains one to six polypeptides that are immunologically related (85,88). In most terminally differentiated mammalian cells, three major lamins (A, B, and C) are commonly expressed in nearly equimolar concentrations. The nuclear lamins have similar sizes, ranging from 60 to 70 kd. Sequence analysis of complementary DNA encoding lamin A and C revealed that these two proteins have identical primary structures up to the amino acid in position 566, and they differ only in the carboxyl terminus (89,90). Six terminal amino acids are unique for lamin C, whereas lamin A is extended at the carboxyl terminus for an additional fragment of 98 residues. Although lamin B gives a tryptic peptide map distinct from that of lamins A and C, it is structurally homologous to the latter. The nuclear lamins share structural homology with intermediate filaments (90). The nuclear lamins form in the interphase nucleus a highly polymerized network anchored at the inner side of the nuclear membrane.

Dramatic structural reorganization of the lamina occurs during mitosis, when it is transiently and reversibly disassembled in the early prophase (85). The lamins become dispersed throughout the cytoplasm and lose their association with chromosomes concomitant with disintegration of the nuclear envelope. When the nuclear envelope reforms during telophase, the lamins progressively polymerize and reassemble. Disassembly and reformation of the nuclear lamina network is mediated by a sequential hyperphosphorylation and dephosphorylation cycles of some of the nuclear lamins (85,91).

Autoantibodies to nuclear lamins have occurred in sera of patients with various autoimmune diseases (92–96). Although the characteristic peripheral staining of the nucleus is indicative for the presence of antilamin autoantibodies, their identity can be conclusively proved by immunoblotting using nuclear pore complexes–lamina fraction or purified nuclear lamins as antigen source. Antilamin antibodies were first described in sporadic cases of a heterogeneous group of patients with linear scleroderma, SLE, and neutropenia (92,93). We have observed such antibodies primarily in AIH (94). In our study comprising 51 patients with AIH and 37 cases of primary biliary cirrhosis (PBC), antilamin antibodies were found in 23% of AIH patients but only in a few cases of PBC. This low incidence in PBC was reminiscent of the sporadic occurrence of antilamin antibodies in systemic rheumatic disease. The antilamin seropositivity was ascertained by immunoblotting after one- and two-dimensional separation of antigens. The use of stringent conditions during immunoblotting allowed detection of high-affinity antibodies and eliminated the risk of the overestimation of positivity. AIH sera reacted preferentially with lamins A and C. Antibodies directed against all three lamins or against lamin B occurred less frequently. Antilamin antibodies in AIH were restricted to the patients with active disease. The relatively high frequency of antilamin antibodies in AIH was confirmed by other groups (95,96).

The occurrence of antibodies directed against the minor constituent of nuclear lamina, lamin B_2, in sera of AIH patients has been described (97). The reactivity with four distinct domains of lamin B_2 was examined in detail and compared with that exhibited by sera of patients with autoimmune rheumatic disorders. Most of the tested AIH sera were reactive with at least two domains of lamin B_2, including the carboxyl-terminal region. None of them recognized a fragment encompassing the nuclear localization sequence that is less conserved and rather specific for lamin B_2. Sera of patients with autoimmune rheumatic diseases mostly recognized only one domain. Some differences in the specificity between distinct disorders could be observed. These findings suggest that recognition of particular lamin protein domains by antibodies in patients' sera differs with pathology.

Microsomal Autoantigens in Autoimmune Hepatitis

In 1973, Rizetto et al. (98) described an autoantibody in sera from a small percentage of AIH patients that stained the proximal renal tubes and the cytoplasm of hepatocytes in rat kidney and liver sections and was detected by indirect immunofluorescence (Fig. 15.2D). The staining pattern differed from that of antimitochondrial antibodies (AMAs). Because the highest reactivity was observed with microsomal fraction,

these autoantibodies were defined as liver-kidney microsomal (LKM) antibodies (98–101). The reactive antigen was characterized by immunoblotting using proteins of isolated human microsomes as antigen. Analysis of immunoblots performed with AIH sera that were LKM positive by immunofluorescence revealed a 50-kd protein as the major antigen (102,103). Further screening of human liver cDNA libraries combined with immunoprecipitation led to identification of human cytochrome P450 2D6 (CYP2D6) as the main 50-kd microsomal antigen (104,105), called LKM1. Incubation of native sera or affinity-purified LKM1 antibodies with isolated microsomes or recombinant cytochrome CYP2D6 protein resulted in the inhibition of enzymatic activity, thereby indicating that circulating LKM1 antibodies may impair enzyme function (106).

CYP2D6 is a member of a drug-metabolizing enzyme superfamily (107,108) that is responsible for the metabolism of different types of agents, including β-blocking agents, antidepressants, antiarrhythmics, and antihypertensive drugs such as debrisoquine. Human liver CYP2D6 is an enzyme form that metabolizes debrisoquine and several other drugs subjected to the same genetic polymorphism. About 10% of the Caucasian population fails to express CYP2D6 in the liver and consequently shows a deficiency for drug metabolism mediated by that enzyme. The lack of enzyme expression was caused by defective alleles (109).

The immunodominant B-cell epitope of CYP2D6 has been localized within a linear sequence of 8 amino acids in the CYP2D6 molecule (110). The sequence of this epitope is highly conserved for class 2D cytochrome P450s. LKM1 antibodies are restricted to the immunoglobulin subclasses IgG1 and IgG4. Anti-CYP2D6 antibodies were found in patients with idiopathic AIH.

Although the CYP2D6 is mainly localized in microsomes, there are some lines of evidence that this enzyme is at least partially anchored in the hepatocyte plasma membrane. First indications came from Lenzi et al. (111), who showed that a LKM-positive AIH serum stained by immunofluorescence methods the surface of isolated rabbit hepatocytes. A similar observation for human hepatocytes was made by Loeper et al. (112,113). Moreover, they isolated the plasma membrane fraction from human hepatocytes and examined it for the presence of CYPs. The specific content of CYP in plasma membrane was 9% of that in microsomes. The anchorage of a small portion of cellular CYPs in the human hepatocyte plasma membrane seems to be specific and not caused by microsomal contamination. The activities of two specific markers of the endoplasmatic reticulum—NADPH-cytochrome *c* reductase and glucose-6-phosphatase—in the plasma membrane fraction were less than 1% of their respective specific activities in microsomal fraction, excluding microsomal contamination. Several CYP forms (1A2, 2C, 2D6, 2E1, and 3A4) were detected among plasma membrane proteins by immunoblotting techniques. The CYP in the plasma membrane fraction was complete, consisting of its protein and its heme moiety.

From the immunofluorescence assay and immunoperoxidase labeling, it is clear that at least some CYP epitopes are exposed on the outer surface of plasma membrane. Anti-LKM human sera and anti-CYP2D6 rabbit polyclonal antibodies recognize plasma membrane CYP2D6 *in situ*. This finding may have important implications. Functional plasma membrane CYPs may form reactive metabolites that covalently bind to these and other proteins. CYP modified by the covalent binding of reactive metabolites represents a new antigen and may trigger the formation of anti-CYP antibodies. Because of the CYP2D6 epitope presentation on the outer surface of hepatocytes, specific autoantibodies may be generated that participate with cytotoxic T cells in the immunologic destruction of hepatocytes in some forms of AIH.

Although CYP2D6 is the most prevalent antigen recognized by anti-LKM1 sera, a minor group of patients react with microsomal antigens that differ from CYP2D6. Another anti-LKM autoantibody exhibiting a slightly different staining pattern in liver and kidney sections was found in hepatitis cases caused by the diuretic drug ticrynafen (tienilic acid) (114,115) and was designated LKM2. This type of drug-induced hepatitis is mainly restricted to some countries, such as France and the United States, in which ticrynafen has been prescribed. These anti-LKM2 autoantibodies are directed against a human liver CYP2C9, which selectively transforms tienilic acid into a reactive metabolite. After withdrawal of the drug, the liver disease decreased, and the titers of anti-LKM2 antibodies markedly declined.

LKM3 antibodies, originally reported to occur in about 10% of patients with chronic hepatitis D (116), were also found in some patients with AIH (117). Anti-LKM3 antibodies recognize an antigen of about 55-kd and pI 8.0, which is expressed at low concentration in neonatal liver and which is easily inducible by agents such as phenobarbital and dioxin. Screening of a human liver cDNA library led to identification of cDNA encoding the LKM3 antigen (117). The sequence of this cDNA was highly homologous with that of uridine diphosphate-glucoronosyltransferase (UGT) of family 1. Characterization of the autoepitope revealed that most anti-LKM3–positive sera react with the carboxyl-terminal part of the enzyme (118). The carboxyl-terminal domain of UGT is encoded by exons 2 through 5, which are common to all members of family 1 UGTs. Using recombinant UGT expressed in a baculovirus system, an enzyme immunoassay was developed. Determination of the autoantibodies' specificity revealed that anti-UGT antibodies in chronic hepatitis D differ from those of the genuine AIH in that the titers are lower and that they recognize different epitopes. Anti-UGT–positive AIH sera react exclusively with family 1 UGTs. A subset of AIH patients develop autoantibodies to both microsomal antigens: CYP2D6 and UGT.

Another type of antimicrosomal autoantibody, reacting with the endoplasmic reticulum of liver preparations but not with that of kidney sections and therefore called antiliver microsome (anti-LM) autoantibody, was detected in patients with dihydralazine-induced hepatitis (119). These antibodies

are directed against human liver CYP1A2, an enzyme involved in the metabolic activation of dihydralazine. The anti-LKM antibodies associated with ethanol-induced hepatitis recognize the hydroxyethyl radical-CYP2E1 adducts (120).

Soluble Liver Antigen

Many AIH patients have antibodies directed against cytosolic SLA (121). SLA was originally defined as a nonspecies, non–organ-specific antigen despite its highest concentration in liver and kidney (121). According to the published protocol (121,122), it was isolated from rat liver as a 150,000 g supernatant after extensive ultracentrifugation of a homogenate. SLA is a heterogeneous fraction consisting of at least a hundred extremely soluble proteins. Wächter et al. (123) reported that anti-SLA–positive AIH sera preferentially react with cytokeratins 8 and 18 and proposed that both cytokeratins are target antigens in SLA. Cytokeratins are expressed in a cell- and tissue-specific manner (124,125), and as members of an intermediate-filament family, they are poorly soluble. This property is routinely used for isolation of cytokeratins, which are readily pelleted after centrifugation for 20 minutes at 3500 g, even in the presence of nonionic detergents and high salt concentrations. Because the protocol used for isolation of SLA is based on extensive ultracentrifugation steps that allow the separation of organelles and cytoskeleton from soluble cytosol proteins, it seems unlikely that cytokeratins represent the target antigens. We characterized SLA and showed that the major antigen belongs to the superfamily of glutathione *S*-transferase (GST) (126), multifunctional enzymes with similar or overlapping specificities (127,128). By microsequencing of the reactive protein spots, at least three distinct GST subunits (Ya, Yb_1, and Yc) between 25 and 27-kd were identified as antigen targets. Further analysis with affinity purified GSTs revealed that 25 of 31 SLA-positive AIH cases reacted with the enzyme. When tested with isolated GSTs, the various titers of antibodies and different reactivities of individual sera toward distinct GST subtypes became apparent. The differential reactivity of the sera with subunits suggests that autoantibodies can distinguish epitopes characteristic for distinct variants and offers evidence for determinant spreading rather that epitope cross-reactivity.

GSTs constitute more than 5% of the total liver proteins (127) that can be extracted from the rat liver and are extremely soluble. GSTs are involved in hepatic detoxification of cytotoxic compounds and carcinogens, and they act as carrier proteins in biliary secretion (127,128). Aside from the catalytic role, the enzymes may be considered as binding proteins that prevent genotoxic agents from interacting with DNA. Mammalian GSTs are encoded by at least four gene families; cytosolic GSTs appear to be products of three families, whereas microsomal GST is genetically distinct (127). The importance of this enzyme family in the function of healthy liver makes them an interesting target for a liver disease–specific antigen. It cannot be excluded that these enzymes become exposed or released or that there is an alteration in synthesis in response to liver damage during initiation of disease (129).

Diagnostic Approach

No disease manifestations are pathognomonic of AIH, and its diagnosis requires the confident exclusion of other conditions. AIH has been defined as an unresolved, predominantly periportal hepatitis that is usually accompanied by hypergammaglobulinemia and tissue autoantibodies and that is responsive to immunosuppressive therapy in most instances. Because these criteria do not enable a simple diagnosis of AIH, the International Hepatitis Group put forward a set of findings necessary for the diagnosis of AIH (46) (Table 15.1).

Therapy

Not all patients with a diagnosis of AIH need therapy. Treatment benefits have been established for patients with severe disease, but treatment guidelines are lacking for individuals with minimal or no symptoms. Prednisolone with or without azathioprine is effective in the management of AIH. Combination therapy is generally preferred, because it is associated with fewer side effects than monotherapy with prednisolone. Basically, treatment starts with 30 mg of prednisolone plus 50 mg of azathioprine daily, and the dosage is then reduced according to the liver function tests. In the monotherapy regimen, prednisolone is started at 60 mg daily. With this regimen, remission is achieved in two thirds of patients after 2 years of therapy.

PRIMARY BILIARY CIRRHOSIS

PBC is a chronic liver disease that occurs predominantly in women. It is characterized by obliteration of small intrahepatic bile ducts and portal inflammation leading to fibrosis and eventually to cirrhosis (130–133) (Fig. 15.3). Although the disease is far less prevalent than viral and alcoholic liver disorders, it represents one of the most common indications for liver transplantation worldwide.

The cause of PBC remains obscure. It may develop in a susceptible individual after exposure to an environmental trigger. Common presenting symptoms are pruritus, nonspecific complaints such as lethargy, and right upper quadrant pain; less frequently, patients have signs of hepatic failure, such as ascites, jaundice, variceal and bleeding. PBC evolves through four stages: an asymptomatic state in patients with normal liver function test results (PBC is suspected by demonstration of antimitochrondrial antibodies), asymptomatic patients with abnormal liver test results, symptomatic patients, and those with decompensated disease.

Since PBC was first regarded as an immune-mediated disease, research has focused on the role of autoantibodies in the pathogenesis of this enigmatic disease (134–136). Using complement fixation and then indirect immunofluorescence tests, it was recognized that almost all patients with PBC pro-

TABLE 15.1. *Diagnostic criteria for autoimmune hepatitis*

Definite autoimmune hepatitis	Probable autoimmune hepatitis
Normal serum alpha$_1$-antitrypsin,[a] copper and ceruloplasmin[a] levels	Abnormal serum copper or ceruloplasmin levels but Wilson's disease excluded
Seronegativity of IgM anti-HAV, HBsAg,[a] IgM anti-HBc,[a] and anti-HCV[a] (RIBA nonreactive)	Anti-HCV may be present but not active true infection
Seronegativity for cytomegalovirus and Epstein–Barr virus	Same requirement
No parenteral blood exposure	Same requirement
Average ethanol ingestion <35 g daily for men and 25 g for women	<50 g daily for men and 40 g daily for women
No recent use of hepatotoxic drugs	Recent use but active disease after drug withdrawal
Any serum aminotransferase[a] abnormality (must exceed alkaline phosphatase[a] elevation)	Same requirement
Gamma globulin,[a] IgG, or total globulin level >1.5 times normal	Any elevation acceptable
SMA,[a] ANA,[a] or anti-LKM1[a] >1:80 in adults or 1:20 in children	Titers at least 1:40 in adults and 1:10 in children
Liver biopsy examination[a] showing moderate-to-severe piecemeal necrosis with or without lobular hepatitis or central–portal bridging necrosis	Seronegativity acceptable if other liver-related autoantibodies are present
No biliary lesions, granulomas, copper deposition, or other features suggesting different diagnosis	Same histologic requirements

ANA, antinuclear antibodies; HBsAG, hepatitis B surface antigen; HCV, hepatitis C virus; LKM1, liver–kidney microsomal antigen; SMA, smooth muscle antigens.

[a] Denotes most cost-effective tests for diagnosis in adults.

duce autoantibodies that are not organ or species specific and that are directed against mitochondrial antigens (134–136). Later, these PBC-associated AMAs were shown to react with trypsin-sensitive antigens on the inner mitochondrial membrane (135); they were designated as M2 antigens. Moreover, sera of approximately 50% of PBC patients contain antibodies to nuclear structures. When viewed by means of indirect immunofluorescence microscopy, two distinct antibody staining patterns can be identified, those showing staining of the nuclear periphery (rimlike pattern) and another group exhibiting multiple nuclear dots.

FIG. 15.3. Histologic appearance of a liver biopsy sample from a patient with primary biliary cirrhosis.

Antimitochondrial Antibodies

AMAs provide the major criterion in the diagnosis of PBC. They are routinely detected by indirect immunofluorescence tests and can be further characterized by immunoblotting and ELISA. Immunofluorescence is typically performed using Hep-2 cells or commercially available rodent tissue sections (Fig. 15.4). The indirect immunofluorescence test for AMA have serious drawbacks. While recognizing the positivity or negativity for AMA, it tells nothing about the antigen targets. In some cases, interpretation of staining patterns is difficult because of nonspecific findings or the presence of other antibodies, resulting in overlapping patterns. Other anticytoplasmatic antibodies that are frequently confused with AMA are antibodies to CYP2D6. To unequivocally identify the autoantibodies, an immunoblotting test using mitochondrial preparations is the method of choice. Immunoblotting experiments demonstrate that there is usually reactivity with one or more mitochondrial autoantigens; a 74-kd protein is most frequently recognized (137,138). Other antigens of different molecular masses (i.e., 56, 48, 41, and 36-kd) were less common.

In the late 1980s, the cloning and sequencing of a cDNA selected by screening a rat liver cDNA library with sera from PBC patients led to identification of the mitochondrial antigens (139–142). The targets of AMAs are members of a functionally related enzyme family, the 2-oxo-acid dehydrogenase complex (2-OADC), that includes the E2 subunits of the pyruvate dehydrogenase complex (PDC), the branched-chain 2-oxo-acid dehydrogenase complex (BCOADC) and

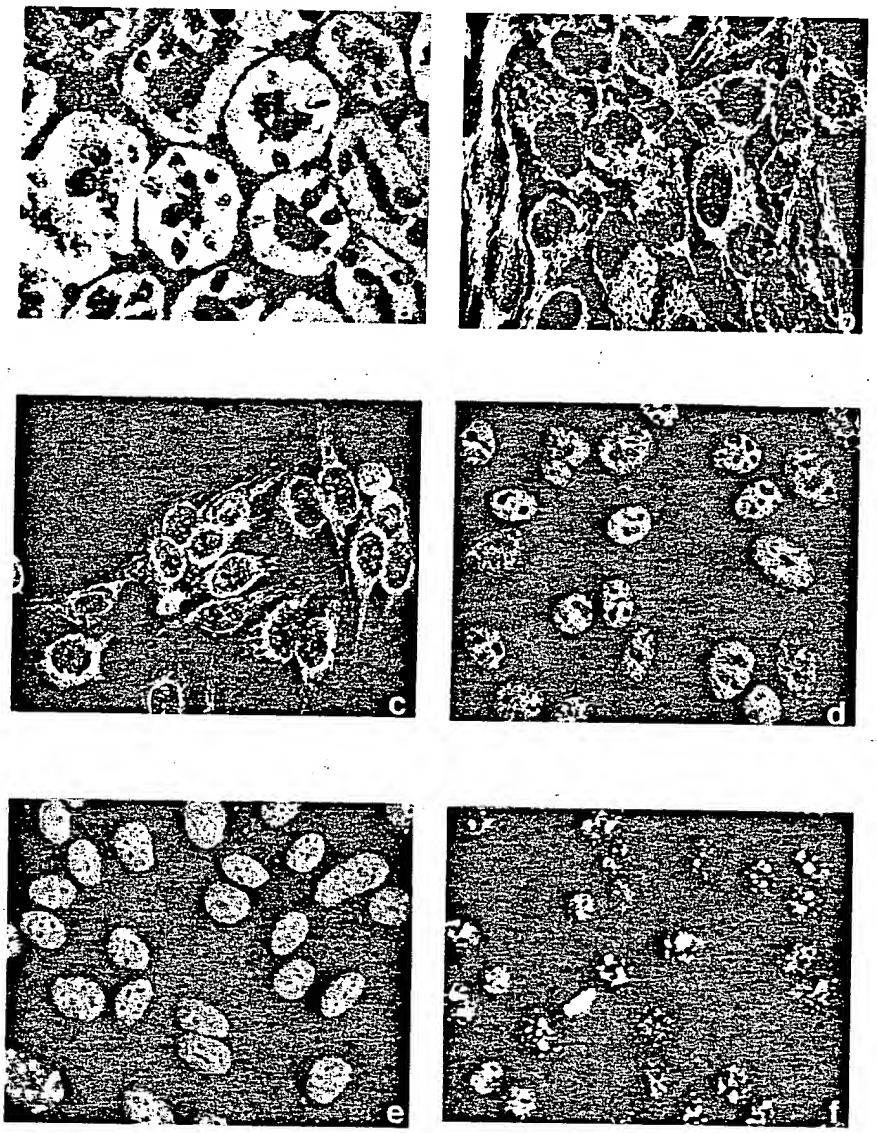

FIG. 15.4. Immunofluorescence patterns displayed by sera from patients with primary biliary cirrhosis (PBC). For detection of autoantibodies, rodent tissue preparations **(A)** or HEp-2 cells **(B–F)** were used. **A, B:** Antimitochondrial antibodies. **C:** Staining pattern shown by anti-gp210–specific serum. **D:** Speckled staining pattern. **E:** Nuclear dots stained by anti-Sp-100–positive PBC serum. **F:** Anticentromere staining.

the 2-oxoglutarate dehydrogenase complex (2-OGDC). Some sera also recognize the E1α subunit of the pyruvate dehydrogenase complex and protein X. Each of the enzyme complexes consists of three subunits E1, E2, and E3. These three 2-OADCs have key roles in intermediary metabolism, and their E2 components are highly conserved in evolution. The E2 components of different complexes exhibit a high degree of structural homology. PBC sera do not always react with all mitochondrial autoantigens; the pattern of reactivity tends to differ from patient to patient. The specificity of AMAs displayed by PBC sera is summarized in Table 15.2. It is

TABLE 15.2. *Specificity of antimitochondrial antibodies in primary biliary cirrhosis sera*

Antigen	Molecular weight	Reactivity (%)
PDC-E2	74	92
BCOADC-E2	56	54
OGDC-E2	48	66
Protein X	52	?
PDC-E1α	41	66
PDC-E1β	36	1–7

remarkable that the PBC sera predominantly recognize the E2 component of each enzyme complex, which is a lipoamide acetyltransferase for the PDC and BCOADC and a succinyl transferase for the OGDC, probably because of their homology. Three-dimensional structure of the E2 component of PDC offered insights into the mode of autoimmune recognition (143).

In addition to indirect immunofluorescence and immunoblotting, ELISA using recombinant mitochondrial antigens has been successfully employed in the serologic diagnosis of PBC (144,145). ELISA permits screening of a large number of specimens and has proved to be highly sensitive and specific. Moreover, the use of "designer molecules," different antigens exposed as single hybrid proteins, permits testing of multiple sera for antigen and epitope specificity in a highly efficient way (145).

There are, however, several caveats in AMA testing. Almost 10% of PBC patients are negative for AMAs; for them, the diagnosis still rests on the clinical features, biochemistry, and histologic findings. Neither positivity for AMA nor its titer relate to the severity of disease and cannot be applied in the follow-up of the clinical course.

Antimitochondrial Antibodies and Disease

Despite characterization of the mitochondrial autoantigens, it remains difficult to reconcile their role within the disease process, a granulomatous destruction that is limited to the small intrahepatic bile ducts. Several hypotheses have been put forward to explain the possible participation of AMAs in the pathogenesis of PBC. One possibility includes the abnormal expression of mitochondrial proteins on bile duct epithelial cells, and another postulates an abnormal translocation of the 2-OADC enzymes. The most attractive hypothesis favors abnormal expression of pyruvate dehydrogenase or a cross-reactive molecule in the involved segments of the bile ducts.

Antibodies Against Constituents of the Nuclear Pores

Nuclear pore complexes are integral components of a nuclear envelope, a proteinaceous structure separating the nucleus from the cytoplasm (146). The nuclear pores, morphologically and functionally distinct from other components of the nuclear envelope, consist of several proteins, with glycoproteins being the most frequent constituents (147,148). PBC sera exhibiting a rimlike staining pattern can recognize proteins of the nuclear envelope (149–151). About one third of PBC patients develop autoantibodies against glycoprotein 210 (gp210), an integral member of the nuclear pores (152,153). "Mature" gp210 is composed of three main domains (154–157). A large 1,783–amino acid amino-terminal domain is located in the perinuclear space between the outer and inner nuclear membrane. A short hydrophobic segment consisting of 20 amino acids spans the outer nuclear membrane, and the 58–amino acid carboxyl-terminal tail domain faces the cytoplasm. gp210 contains asparagine-linked high mannose-type oligosaccharides (154,155). The carbohydrate residues are not randomly distributed within the gp210 molecule, but they are restricted to its amino-terminal part (156,157). Autoantibodies against gp210 recognize at least two different epitopes. About one third of anti-gp210–positive PBC sera react with a short 15–amino acid fragment within the carboxyl-terminal part of gp210 (158). Most gp210-seropositive PBC sera recognize an epitope located within the large glycosylated luminal amino-terminal domain (153,159). Carbohydrate residues are an essential part of the epitope. About 65% of anti-gp210–positive PBC sera lose their affinity for gp210 on its enzymatic deglycosylation (153,159).

The complete gp210 sequence has been determined for the rat (155) but not for humans. A comparison of a short, partial sequence of human gp210 revealed considerable differences from that of rats. The cDNA coding for rat gp210 was in the past cloned into the expression vector, allowing generation of the recombinant protein in *Escherichia coli.* Unfortunately, the bacterially expressed gp210 recombinant protein lacks posttranslational modifications and therefore is not suitable for application as an antigen for screening anti-gp210 antibodies. It remains unknown whether the sequence encompassing the epitopes exhibits homology between humans and rats.

Anti-gp210 antibodies seem to be highly specific for PBC, and their detection may be useful, especially in diagnosing individuals without AMAs. Antibodies against p62 protein, another component of nuclear pores, have been described in about 30% of PBC sera (160). The p62 protein belongs to a group of mammalian nuclear pore glycoproteins that are modified at approximately 10 to 20 sites with *O*-linked *N*-acetylglucosamine (161). These proteins are involved in nucleocytoplasmic transport. p62 represents the best characterized member of this family. The *N*-acetylglucosamine residues are attached to the serine/threonine stretch in the central part of protein. Deglycosylation of p62 did not affect its reactivity with PBC sera, indicating that recognized sugar moieties are not an integral part of the immunodeterminant. These antibodies, also detected by other groups, appear to represent a distinct entity and do not colocalize with anti-gp210 (160,162–164).

Anti-gp210– and anti-p62–positive PBC patients show features, in terms of clinical data, biochemistry, and morphology, similar to those of PBC patients who are solely positive for AMAs (165). Several studies have shown that these antibodies might be of special value in the diagnosis of PBC in patients who are seronegative for AMAs (165).

Antibodies Showing Nuclear Dot Staining

ANAs featuring the nuclear dot pattern have been widely observed in PBC sera (166). This staining pattern is defined by multiple nuclear dots of variable size and with wide distribution over the cell nucleus, although sparing the nucleolar region. This pattern is characteristic, but it may be erroneously

classified as that of ACAs, which occur in roughly 15% of PBC sera. However, the staining characteristics are quite different. First, centromere antigens are mostly of uniform size, and nuclear dots vary in size and number in the individual cell. Second, antibodies giving the nuclear dot pattern in distinction to ACAs give the same staining pattern of the interphase and the mitotic cells.

Antibodies to Sp-100

Sp-100 was identified as a target antigen of antibodies, showing dotlike intranuclear localization by means of immunofluorescence microscopy (167). It is an acidic nuclear protein (pI 5.2), with an electrophoretic mobility corresponding to 100 kd. Isolation and cloning of the Sp-100 full-length cDNA revealed a sequence encoding a protein of 480 amino acids with a predicted molecular mass of 50-kd (168). The Sp-100 sequence demonstrates two regions with similarities to known proteins, one to the antigen binding site of the human MHC class 1 molecules and another to several transcriptional regulatory factors, including the human immunodeficiency virus (HIV) nef-1 protein. Determination of epitope mapping with different truncated Sp-100 recombinant proteins revealed at least three nonoverlapping major autoantigenic determinants that were recognized by most PBC sera. One domain, which contains the sequence similarity with the HIV nef-1 protein, was recognized by all anti-Sp-100–positive sera (169). The biologic function of Sp-100 protein is unknown. Infection of Hep-2 cells with influenza A virus or mitogenic stimulation of peripheral blood lymphocytes affected expression of the Sp-100 protein. Notable enhancement of the Sp-100–specific mRNA and of protein expression was observed during cultivation of human cells in the presence of three different types of interferons (IFNs), whereas no such effect occurred after treatment with TNF-α (170). These findings characterize Sp-100 protein as a new member of cytokine-modulated proteins.

Another protein giving the nuclear dot pattern, the promyelocytic leukemia (PML) antigen, colocalizes with Sp-100 and shows a disease specificity similar to that of Sp-100 antibodies (171). The PML autoantigen, discovered in the context of leukemic transformation, is expressed in the form of many COOH-terminally spliced variants and is enhanced by IFNs. Both proteins are covalently modified by PIC1/SUMO-1, a small ubiquitin-like protein (172). This modification may play a regulatory role in the structure and function of these autoantigens.

Anticentromere Antibodies

The frequency of ACAs in PBC is 10% to 50%, depending on the tests used for their determination (74,173,174). In our experience, ACAs are observed in about 15% of PBC sera. Detailed information on the structure and function of autoantigens is given the "Antibodies to Nonhistone Proteins" section (page 234).

Diagnostic Approach

The diagnosis of PBC is normally straightforward. It includes a cholestatic laboratory profile, diagnostic or compatible morphology, and a positive test for AMA and sometimes for gp210.

Therapy

Almost all immunosuppressive drugs have been tried in controlled trials of medical therapy for PBC. Steroids led to the biochemical and morphologic improvements but also to considerable side effects. Other drugs such as azathioprine, cyclosporin, and methotrexate have shown marginal efficacy in routine therapy. However, several placebo-controlled trials have shown that ursodeoxycholic acid (UDCA), a hydrophilic bile acid, improves the symptoms, biochemistry, and histology. Use of this drug prolongs survival and retards liver transplantation. UDCA is the drug of choice for treating PBC, but liver transplantation has a definite place in the treatment of the end-stage PBC, with 5-year survival rates approaching 80%.

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is the second most common chronic inflammatory disorder, after rheumatoid arthritis, and comprises at least two entities: Crohn's disease (CD) and ulcerative colitis (UC). CD and UC are differentiated by differences in clinical presentation, course, diagnostic criteria, response to treatment options, and prognosis. The available data suggest that the diseases develop on different, heterogeneous polygenic backgrounds under the influence of acquired or environmental factors, which have not been clearly identified. However, because both diseases share a number of epidemiologic, pathophysiologic, macroscopic, and microscopic features and because no standard has been established for differentiating them, discrimination continues to be based on a combination of clinical and diagnostic observations (175,176).

CD is a chronic, relapsing disease that can affect the entire gastrointestinal tract and is characterized by a focal, asymmetric, transmural, and occasionally granulomatous inflammation. It has the potential for systemic and extraintestinal complications. CD is a lifetime affliction and recurs after resective surgery. In UC, the relapsing inflammatory process is confined to the mucosa and superficial submucosa of the colon and rectum, extending typically in a continuous manner proximally from the rectum. UC is a surgically curable disease.

Sulphasalazine or 5-aminosalicylic acid, glucocorticoids, and immunosuppressive drugs are standard therapies for IBD. Advances in understanding the pathogenetic mechanisms of CD and UC led to the introduction of antiinflammatory and immunosuppressive strategies that may challenge the application of current treatment regimens in the near future (177).

Epidemiology

The incidence and prevalence rates of IBD vary substantially, depending on differences in racial and ethnic groups, and they are associated with the socioeconomic development, which points to the importance of genetic and environmental factors in the pathogenesis of CD and UC. The incidence of IBD is highest among whites, relatively low in blacks, and lowest among Asians (178). Jews have increased incidence and prevalence rates for IBD compared with other ethnic groups in the same locations. A North–South gradient has been observed, with higher rates found in Northern European countries. Overall, the prevalence of CD has increased in Western Europe and the United States and is estimated at 30 to 50 cases per 100,000 persons, with an incidence of 1 to 10 cases per 100,000 persons. The incidence rate of UC is 2 to 6 cases per 100,000 persons, with a prevalence that has remained relatively constant over the last five decades at 40 to 100 cases per 100,000 persons (179). The frequencies of UC and CD in men and women are essentially equivalent, with differences found among racial and ethnic groups. CD and UC can manifest at any age but is most common in teenagers and young adults, and a second smaller increase in incidence occurs in the fifth and sixth decades.

Genetics

Family and epidemiologic studies strongly support the assumption that neither CD nor UC has a simple mendelian pattern of recessive inheritance, even with variable penetrance, but that both diseases are related polygenic disorders. The higher concordance rates of IBD, particularly CD, in identical compared with nonidentical twins; the 10-fold increased risk of IBD among relatives with CD and UC, particularly siblings, versus spouses; and differences in disease prevalence in different ethnic groups and migrants have provided strong evidence that genetic predisposition is important in the pathogenesis of IBD (180–182). Susceptibility to CD in families may involve a gene that is imprinted, because there is a higher risk of transmission from the mother than from the father (183). Concordance for disease type (i.e., inflammatory, stricturing, or fistulizing) and site has been observed in affected family members with CD (184,185). Evidence for the existence of genetic anticipation in CD has been provided by pointing out that the age at onset is lower and disease more extensive in siblings than in offspring of affected parent-child pairs (186,187). Later findings of clinical differences between familial and sporadic CD need to be further substantiated (188).

Molecular genetic studies have provided novel information regarding susceptibility genes involved in the pathogenesis of CD and UC. Genes encoding MHC antigens, cytokines, and adhesion molecules have been analyzed. Associations of haplotypes of the MHC as determinants of disease susceptibility and behavior in IBD have been extensively investigated, as in many other diseases with a suspected autoimmune origin or pathogenesis, and seem to be more important in UC than in CD. HLA class II associations with UC are complex, and separate alleles confer susceptibility or resistance. For Japanese and Jewish patients, several studies have shown an increased frequency with haplotype DR2, but for Caucasian populations, the results are more conflicting. The equivocal results with HLA-DR2 appear to be caused by positively and negatively associated subspecifities in this group. HLA-DRB1 may even predict extensive disease or extraintestinal manifestations in UC. The incidence of DRB1*15 allele was increased among UC patients having a positive family history (189).

Hugot et al. reported the first genome-wide screening for susceptibility genes in IBD (190). A locus on chromosome 16, designated IBD1, was identified that could account for at least 10% of susceptibility to CD. Putative candidate genes included in this region are the interleukin-4 receptor and CD11 integrin. A subsequent two-stage genome-wide search for susceptibility genes revealed striking evidence for linkage between IBD and regions on chromosomes 12, 7, and 3. Individual markers on chromosomes 2 and 6 were linked with susceptibility to UC, but not to CD. These data have provided the most rigorous evidence that CD and UC are related polygenic disorders, sharing some susceptibility genes (191). This idea is further strengthened by the finding that no single standard differentiates CD from UC. Strong positional candidates on chromosomes 7 are hepatocyte growth factor, epidermal growth factor receptor, and *MUC3*, a gene encoding the protein backbone of an intestinal secretory mucin. The region on chromosome 3 includes the $G\alpha_{i2}$ gene encoding a subunit of an inhibitory guanine nucleotide–binding protein. Knockout mice deficient for $G\alpha_{i2}$ develop a lethal diffuse colitis, complicated by adenocarcinoma of the colon (192).

Genetic heterogeneity and subgrouping of IBD patients has been advocated by analysis of genetic and subclinical markers. Perinuclear antineutrophil cytoplasmic antibodies (pANCAs) are the most established subclinical markers in IBD, with a high degree of specificity for UC and associated primary sclerosing cholangitis (PSC) compared with CD or other colitides (193). These antibodies are directed against granule proteins of neutrophils such as proteinase-3, myeloperoxidase, cathepsin G, elastase, lactoferrin, or bactericidal/permeability-increasing protein, and they probably reflect a genetic susceptibility because of the finding of increased levels in unaffected relatives of patients with UC (194–196). The pANCAs in the serum of patients with UC may reflect a genetically determined dysregulation of immunoglobulin production. Several findings argue against a pathogenic role of pANCA in UC, but demonstration that these antibodies also recognize a cytoplasmic antigen expressed in the neuroendocrine cells of the pancreas, retinal cells, and nonepithelial cells of the gastrointestinal tract, skin, and lung could point to an autoimmune-mediated colonic inflammation elicited by these autoantibodies (197,198).

Antibodies to the mannan portion of yeast, anti-*Saccharomyces cerevisiae* antibodies (ASCAs), occur in up to 70% of patients with CD and in 20% of their healthy first-degree relatives but only 5% of patients with UC (199). ASCA combined with ANCA may help differentiate UC from CD, and heterogeneity may help to stratify patients with CD (200). Goblet cell antibodies (GABs) have a prevalence of approximately 30% among patients with CD and UC. The high prevalence among first-degree relatives suggest that GABs may represent another marker characterizing susceptibility to IBD (201).

Genetic polymorphism in the gene coding for the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1RA) has been associated with UC by demonstrating an overexpression of its allele 2. This has been interpreted as a possible genetic predisposition for severity of the inflammatory course but the concept has been challenged by others (202–204). A mucosal imbalance of the proinflammatory cytokine IL-1 and IL-1RA has been demonstrated in IBD, supporting the pathogenic importance of this antagonizing system for the perpetuation of intestinal inflammation (205). Associations of polymorphisms of TNF microsatellites and intercellular adhesion molecule 1 gene with CD have been inconclusive (206–208).

Impairment of the epithelial barrier function leading to increased intestinal permeability against antigens and proinflammatory molecules has been found by many groups in patients with CD and described as an predictive factor for clinical relapse. However, the role of this epithelial dysfunction as an genetic marker of disease susceptibility remains to be established (209,210). Additional candidate genes for a genetic contribution to IBD are those encoding mucin apoproteins or mucin glycosylating proteins. Alterations in mucin glycoproteins have been found consistently in patients with UC and unaffected identical twins of patients with UC but not in patients with CD (211).

Models of unrestrained mucosal inflammation in gene-targeted mice provide a novel approach to investigate susceptibility genes for IBD. Chronic intestinal inflammation in mice results from deletions of the T-cell receptor, MHC class II molecules, IL-2, IL-10, or the G protein $G\alpha_{i2}$, leading to lymphoid abnormalities from abnormal T-cell development or regulation and from cytokine deficiency (212). From these models, the sensitive regulation of the gut-associated lymphoid tissue that requires a delicate balance between response and anergy can be delineated. The enhanced susceptibility of mice deficient in intestinal trefoil factor or transforming growth factor-β (TGF-β) to colonic injury demonstrates the importance of locally produced growth factors to mucosal homeostasis and healing (213,214). Chimeric mice expressing a dominant negative N-cadherin mutant transgene develop colitis, presumably resulting from altered epithelial cell-cell and cell-matrix interactions that cause an influx of foreign antigens across the damaged mucosa (215). None of the current models is the exact representation of human IBD, suggesting that the human disease may not result from a single genetic defect but reflect insults caused by the alterations of various genes (216).

Environmental Risk Factors

Various environmental factors have been considered in the cause of IBD. Smoking is the most extensively studied environmental risk factor, reported to have opposing effects on the disease severity of CD and UC. Smoking has a protective effect on UC but is deleterious for CD, a discrepancy that may help to clarify the pathogenesis of IBD (217,218). The use of nonsteroidal antiinflammatory drugs (NSAIDs) has been associated with an increased risk of flare-ups of colitis and the need for admission to hospital due to IBD (219). Oral contraceptives, refined sugar, domestic hygiene, and perinatal and childhood infections have been associated with IBD, but further evaluation is required to confirm the consistency of and define the strength of these associations (220).

Several circumstances, such as the improvement of IBD by fecal diversion, bowel rest, or an elemental diet and the importance of the intestinal flora for the development of colitis in genetically altered mice, suggest a pathogenetic role of microbial agents or their products in IBD (221,222). Infectious agents under investigation include *Mycobacterium paratuberculosis, Listeria monocytogenes, Streptococcus* spp., and abnormal *E. coli*, but the evidence supporting these bacteria as etiologic factors is inconclusive. Epidemiologic and basic scientific data have led to the hypothesis that CD is a chronic, granulomatous vasculitis with resultant focal ischemia caused by a persistent, perinatal infection of the vascular endothelium with measles virus (223–225). However, the data have not been validated by other groups (226,227).

The consumption of fish has been suggested as a protective dietary factor in IBD. The antiinflammatory omega-3 fatty acids in fish oil may be particularly important, and initial clinical trials with these substances provide evidence of their effectiveness (228). Appendectomy protects against developing UC, apparently because of its important role in antigen presentation and immune activation within the gut-associated lymphoid tissue (229). Data from T-cell receptor (TCR)-α–deficient mice, a genetic model of UC, support the protective role of appendectomy (230).

Pathogenesis and Immune Mechanisms of Inflammatory Bowel Disease

The cause of IBD is unknown, but preferred theories describe pathogenic or resident luminal bacteria that constantly stimulate the mucosal immune system, leading to a chronic inflammatory response on the basis of genetically determined host susceptibility factors (Fig. 15.5). As an initial step in the pathogenesis of IBD, the breakdown of the intestinal mucosal barrier by infections, toxins, or NSAIDs results in the exposure of lamina propria immune cells to luminal bacteria, bacterial products (including superantigens), cell wall components and toxins, dietary antigens, or possibly self-antigens,

Initiating events →	Perpetuating events →	Impaired mucosal balance →	Mediators of tissue damage
Infections	Luminal bacteria	Genetic susceptibility	Cytokines: TNF-α, IFN-γ
Toxins	Bacterial products	CD4+ lymphocytes	Chemokines: IL-8, ENA-78
NSAIDs	Dietary antigens	Imbalance IL-1/IL-1RA	Neutrophils, eosinophils
Multifocal gastrointestinal	Autoantigens	Imbalance Th1/Th2	Macrophages
infarction?	Smoking (CD)	Imbalance pro-/anti-	Eicosanoids
		inflammatory cytokines	Nitrogen and reactive oxygen
		oral tolerance ↓	metabolites
		Epithelial permeability ↑	Proteases
		Mucin abnormality	Complement

FIG. 15.5. Proposed model of pathogenesis of inflammatory bowel disease. (Adapted from Sartor RB. Pathogenesis and immune mechanisms of chronic inflammatory bowel disease. *Am J Gastroenterol* 1997;12:5S–11S.)

which perpetuate the inflammatory process. Macrophages and helper T-lymphocyte subsets become activated and release proinflammatory cytokines and chemokines that recruit other inflammatory cells, such as monocytes, lymphocytes, neutrophils, and eosinophils to the intestinal process. A self-perpetuating inflammatory cascade causes a disturbance between proinflammatory mediators and immunosuppressive mechanisms, leading to the net result of tissue injury and functional abnormalities. CD and UC appear to have its distinct constellation of genetic factors, immunologic characteristics, and pathologic findings (175).

Cell-mediated immune responses to gut antigens are actively prevented by subpopulations of regulatory T cells, mediating their inhibitory function by the cytokines IL-10 and TGF-β (231). Observations suggest that counterregulatory $CD4^+$ T-cell subsets are essential for the development of IBD (232). The demonstration of activated $CD4^+$ lymphocytes in patients with IBD, the symptomatic exacerbation of CD after high-dose IL-2 treatment, the ability of therapeutic agents that modulate lymphocyte activation and of allogenic bone marrow transplantation to ameliorate disease activity, and the apparent requirement for $CD4^+$ T cells in murine models of IBD offer evidence for this hypothesis (233,234). The adoptive transfer of the subpopulation of $CD45RB^{hi}$ $CD4^+$ T cells from normal mice to T-cell–lacking severe combined immunodeficiency (SCID) mice results in the development of a severe and chronic pancolitis in the recipient mice because of the differentiation of transferred cells to type 1 helper T (T_H1) cells, producing increased amounts of IFN-γ, IL-2, and TNF-α (235). $CD4^+$ T_H1 lymphocytes also appear to be the effector cells responsible for the intestinal inflammation in other gene-targeted mice models of IBD and in chronic intestinal lesions of patients with CD, whereas a T_H2 profile of IL-4 and IL-10 production has been shown in early CD and UC (236–239). Lamina propria lymphocytes are expanded oligoclonally in patients with IBD, with the presence of interindividual TCR β-chain complementary determining region-3 patterns, implying an individually distinct selective pressure on the TCR in response to the antigen/MHC complex, which may be determined by the HLA haplotype (240,241). TCR-γδ T cells are also strikingly expanded in the lamina propria of patients with IBD and are a major source of IFN-γ (242). The increased integrin expression on lamina propria lymphocytes may confer the enhanced lymphocyte homing capabilities of these cells to the activated endothelial cells in IBD intestine (243,244).

Controversial reports have been published about the concomitance of active IBD in immunocompromised patients with acquired immunodeficiency syndrome (AIDS). The recurrence of CD after solid organ transplantation despite immunosuppressive therapy and in transplanted bowel in a patient with short-bowel syndrome argue against a major role of T cells in the pathogenesis of IBD (245–247).

In normal intestinal mucosa, most macrophages are resident and display a mature phenotype, whereas in IBD, a monocyte-like ($CD14^+$,$L1^+$) subset of newly recruited cells is present, primed for the production of TNF-α (248). Monocyte-derived macrophages demonstrate upregulation of the costimulatory molecules CD80 and CD86, which are crucial for enhanced antigen-presenting capacity and may be implicated in breaking the immunologic tolerance to luminal antigens in IBD (249). Nuclear factor kappa B (NF-κB), a transcription factor known to regulate the synthesis of inflammatory cytokines such as IL-1, TNF-α, and IL-8, is activated in the macrophages and epithelial cells of inflamed intestinal mucosa (250). Local administration of antisense oligonucleotides to the P65 subunit of NF-κB abrogated clinical and histologic signs of inflammation in mice with transmural granulomatous colitis, pointing to the potential therapeutic utility of this molecular approach in patients with IBD (251).

The presence of neutrophils in intestinal lamina propria because of chemotactic mediators such as IL-8, leukotriene

B_4, platelet-activating factor, and formyl-methionyl-leucyl-phenylalanine is a characteristic finding in IBD lesions (252–256). Through their release of reactive oxygen products and the ensuing oxidation of sulfhydryls, peroxidation of membrane lipids, and degradation of proteins, carbohydrates, hyaluronic acid, and mucin, neutrophils are important contributors to mucosal damage and pathogenesis of IBD (257,258). Proteins of neutrophil granules are found in increased quantities in the lesions and stools of IBD patients (259–261). Neutrophil-derived 5′-adenosine monophosphate is involved in the development of diarrhea by stimulating the chloride secretion of intestinal epithelial cells (262). Evidence exists for the pathogenetic role of eosinophils in IBD, including increased concentrations of eosinophil granule proteins measured in whole-gut lavage fluid (263).

Altered cytokine and chemokine production is a hallmark of IBD, and lymphocyte-dependent models of IBD and cytokine polymorphisms are related to disease severity or the need to surgery. In the normal mucosa, proinflammatory and antiinflammatory cytokines are balanced, whereas in IBD, the production of several proinflammatory cytokines is increased (212,264). The key aggressive regulatory cytokines appear to be IL-1, TNF-α, IL-12, and IFN-γ, whereas IL-4, IL-10, and TGF-β are immunosuppressive (265). IL-12 is expressed by CD intestinal lamina propria mononuclear cells, consistent with the hypothesis of an IL-12–driven expansion of $CD4^+$ T cells of the T_H1 phenotype in the pathogenesis of IBD (266,267). The T_H1 cytokine IFN-γ, which is produced in increased amounts in intestinal IBD lesions, acts directly by injuring epithelial tight junctions and indirectly by activating macrophages to produce inflammatory mediators such as reactive oxygen and nitrogen intermediates and cytokines such IL-1, IL-12, and TNF-α and by induction of MHC class molecules and costimulatory molecules (268). The number of TNF-α–producing cells is also increased in IBD. In CD, TNF-α–positive cells can be detected throughout the mucosa, whereas in UC, only subepithelial macrophages produce TNF-α (269,270). Despite the increased local production of TNF-α, serum concentrations are low, even in active disease (271). A mucosal imbalance of IL-1 and IL-1RA has been described for CD. IL-1 and TNF-α stimulate the production of many proinflammatory mediators, including other cytokines, arachidonic acid metabolites, and proteases, and both are involved in activating T lymphocytes.

The importance of a balanced cytokine production for mucosal immunologic homeostasis is underlined by mouse models in which the immunosuppressive genes for IL-10 and TGF-β have been deleted, producing severe colitis (272,273). IL-10 downregulates the production of multiple proinflammatory cytokines by T lymphocytes and monocytes, and it antagonizes T-cell differentiation toward the T_H1 phenotype (274). Administration of IL-10 significantly inhibited development of colitis in SCID mice restored with $CD45RB^{hi}$ $CD4^+$ T cells and might be beneficial in patients with CD.

Increased amounts of phospholipase A_2–activating protein, the rate-limiting enzyme in the production of arachidonic acid and of arachidonic acid metabolites, have been detected in the inflamed intestinal mucosa of patients with IBD (275). Chemotactic leukotrienes, especially leukotriene B_4, may account for most neutrophil recruitment and activation in IBD, but a pivotal role of leukotriene B_4 in UC was challenged by disappointing trials with inhibitors of its synthesis and action (276). Prostaglandins are produced by the cyclooxygenase (COX) pathway and exhibit proinflammatory and antiinflammatory effects. COX-1 is a constitutive enzyme thought to produce cytoprotective prostaglandins, and COX-2 represents the inducible form of cyclooxygenase, leading to the production of proinflammatory prostaglandins. COX-2 mRNA increases with endoscopic disease activity, but COX-1 mRNA remains unchanged (277). Prostaglandin E_2 may contribute to diarrhea by promoting intestinal electrolyte and fluid secretion. The elevated expression of inducible nitric oxide synthase protein and production of nitric oxide in the mucosa of patients with active CD and UC may provide a protective effect, as shown for acetic acid–induced murine colitis (278,279). Nitrogen and reactive oxygen metabolites may also be mediators of secretory diarrhea in IBD (280).

Nerve-immune interactions may have a significant role in the process of inflammatory changes in IBD. Neuropeptides regulate many of the inflammatory responses and modulate the functional effects of cytokines and other mediators. Substance P has received considerable attention because of its stimulatory effects on various immunocompetent cells, and increased innervation with sustance P– containing nerve fibers has been demonstrated in UC (281). Decreased levels of the immune-inhibitory somatostatin have been described in active IBD (282). Immunoneutralization of nerve growth factor and neurotrophin-3 significantly worsened the chronic trinitrobenzene sulfonic acid (TNBS)–induced colitis in the rat, suggesting an important protective role for neurotrophins in chronic inflammation of the colon (283).

The inflammatory response in IBD involves immune cells, epithelial cells, mesenchymal cells, neurons, and vascular endothelial cells, which are targets of cytokines and other mediators and contribute to the clinical manifestations in IBD (284). In UC, sCD95L or $CD95L^+$ mononuclear cells mediate epithelial apoptosis, which may be involved in the breakdown of the epithelial barrier function, facilitating the invasion of pathogenic microorganisms (285). The protective function of epithelial integrity against chronic enterocolitis is supported by chimeric mice with a dominant negative N-cadherin that develop intestinal inflammation when the crypt architecture is disrupted. Intestinal epithelial cells express the nonpolymorphic antigen-presenting molecule CD1d, and upon exposure to IFN-γ, major histocompatibility complex class II molecules (286,287). Enterocytes may evolve to nonclassic antigen-presenting cells, a development that has been related to the abnormal T-lymphocyte activation patterns in IBD (288). Defective expression of GP180, a novel CD8 ligand

on intestinal epithelial cells in IBD patients, appears to be critical to the failure of intestinal epithelial cells to activate tolerance-inducing $CD8^+$ suppressor T cells. Gut epithelial cells are able to secrete an array of cytokines in a stereotypic response to pathogens, contributing to the cytokine milieu of the lamina propria (289). Enterocytes produce, among others, IL-15, which is a cofactor for IL-12–induced IFN-γ production, and chemotactic epithelial neutrophil-activating peptide 78 (290,291). IL-1, TNF-α, and insulin-like growth factor regulate development of fibrosis by stimulating proliferation of intestinal smooth muscle cells and fibroblasts, contributing to stricture formation and obstruction in CD (292,293).

The assumption that UC and CD are autoimmune diseases has little immunologic documentation. In UC, antitropomyosin antibodies cross-reacting with epithelial cells in the colon, bile ducts, and eye have been described, but this reaction may be of a secondary, nonpathogenic nature (294).

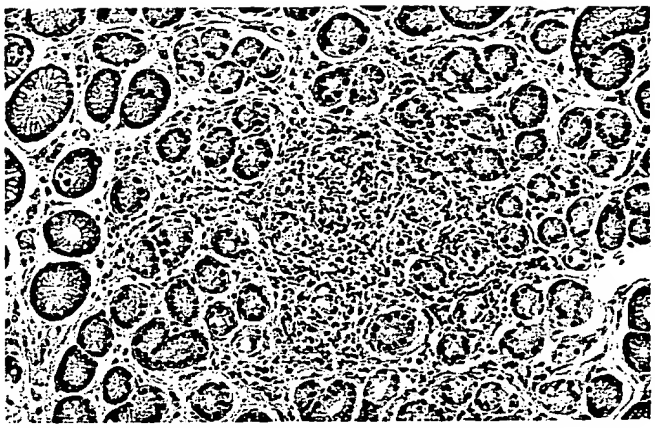

FIG. 15.6. Crohn's disease of the colon as revealed by a small epithelioid granuloma in the center of the micrograph. There is only a mild increase in the number of inflammatory cells. The crypts appear slightly distorted (hematoxylin and eosin stain; original magnification ×400). (Courtesy of G. Oberhuber)

Histopathology

Features that are useful for evaluation of histologic abnormalities in colorectal biopsy specimens from chronic idiopathic IBD patients are mucosal architecture, lamina propria cellularity, neutrophil infiltration, and epithelial irregularity. In most cases, a combination of crypt architectural distortion, decreased crypt density, irregular mucosal surface, transmucosal or discontinuous increased lamina cellularity, and epithelial damages (including flattening, vacuolation, focal cell loss, erosion, and ulceration) is characteristic in chronic idiopathic IBD and much less notable in infective-type colitis or other causes of colorectal inflammation (295,296). Large numbers of neutrophils, especially crypt abscesses, suggest chronic idiopathic IBD. The presence of epithelioid granulomas, defined as a discrete collection of at least five epithelioid cells (activated histiocytes with homogeneous eosinophilic cytoplasm) with or without accompanying multinuclear giant cells, is one of the histopathologic hallmarks of CD, although it is not a sensitive feature, because it occurs in only 18% of biopsy specimens from patients with known CD (297).

The differential diagnosis between UC and CD by means of histopathology depends mainly on the more severe architectural abnormality (i.e., crypt distortion, decreased crypt density, or a frankly villous surface) and greater density and transmucosal distribution of lamina propria cellularity in active UC (Fig. 15.6). Mucin depletion because of a reduction in the number of goblet cells or depleted mucin within cells is specific only for severe UC. In CD, variable, milder, discontinuous architectural abnormalities and epithelioid granulomas are accurate features. Polymorph infiltration is found in UC and CD, although focal infiltration is more often seen in CD (Figs. 15.7 and 15.8).

The histopathologic features found in IBD are characteristic but nonspecific. The histologic appearances of the biopsy specimen alone are not sufficient to predict accurately the final diagnosis in up to 30% of cases of UC and up to 60% of cases of CD. The final diagnosis depends on a combination of clinical, radiologic, and endoscopic findings and on examination of sequential biopsy specimens from multiple sites. A terminal ileal biopsy specimen may be useful if CD is suspected. The designation of indeterminate colitis is extended to cases for which it is not possible to make a distinction between UC and CD (298).

Microscopic colitis defines a clinicopathologic syndrome that manifests as chronic watery diarrhea in the presence of histologic inflammation but absence of definite endoscopic or radiologic abnormality. Lymphocytic and collagenous forms of colitis are included in this syndrome, but IBD, autoimmune disease, graft-versus-host disease, and drug-induced

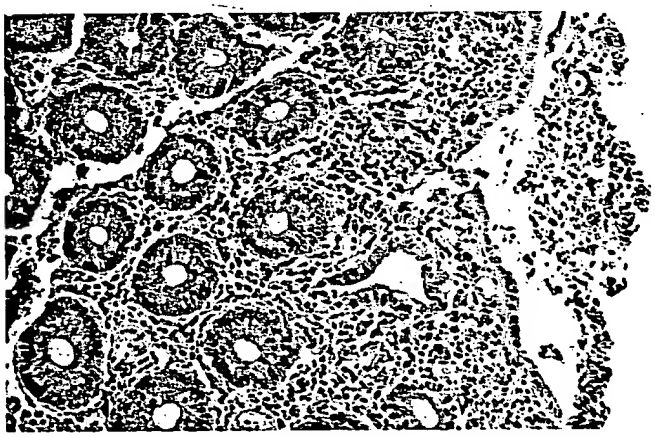

FIG. 15.7. Crohn's disease of the colon as revealed by an aphthous lesion. It is characterized by a subepithelial accumulation of inflammatory cells with accompanying epithelial degeneration, especially at the surface. Notice the cellular debris at the luminal side of the biopsy (hematoxylin and eosin stain; original magnification ×600). (Courtesy of G. Oberhuber)

FIG. 15.8. Mucosal biopsy of the sigmoid colon shows the typical lesion of ulcerative colitis. It is characterized by architectural changes such as crypt distortion and elongation, and the number of goblet cells is decreased. The lamina propria is densely infiltrated by inflammatory cells, with a predominance of plasma cells. Notice the lymphoid aggregate at the basal side of the mucosa (hematoxylin and eosin stain; original magnification ×200). (Courtesy of G. Oberhuber)

inflammation also may present with microscopic colitis. In lymphocytic colitis, the number of intraepithelial lymphocytes is increased from less than 5 per 100 epithelial cells in normal mucosa to more than 20 per 100 (299). In collagenous colitis, the subepithelial collagen layer of normally 3 μm exceeds 10 μm, although it may be patchy and confined to the proximal colon (300,301). Whether lymphocytic and collagenous colitis represent single diseases or reflect the spectrum of a common pathogenesis and have a possible relationship with UC and CD is unclear. Empirical antiinflammatory therapy with 5-aminosalicylic acid may be useful (302).

Clinical Course

Crohn's Disease

Clinical presentation of patients with CD is heterogeneous because of the diversity of intestinal involvement and complications developing during the course of the disease and because of their functional and morphologic impairment after bowel resection. CD may affect any region of the gastrointestinal tract, with rare locations including the oral cavity, esophagus, stomach, and duodenum. In nearly 40% of patients, disease is confined to the small intestine, usually the terminal ileum; in 30%, the large bowel is affected, and in another 30%, the large bowel and small intestine are involved.

Phenotypic classification of CD has been accomplished according to anatomic location (i.e., duodenojejunoileitis, ileitis, ileocolitis, and colonic and perianal disease), disease extent, steroid responsiveness, number of surgical resections, extraintestinal manifestations, and pANCA status. Grouping according to an inflammatory, fibrostenotic, or fistulizing pattern of CD has been proposed, and these groups have different clinical outcomes (303). The Working Party for the World Congress of Gastroenterology in Vienna 1998 developed a simple classification of CD based on the objective variables of age at diagnosis, location, and behavior. Age at diagnosis represents to some extent a genetic component of CD, location delineates disease anatomy, and behavior describes the biology of the disease in terms of the occurrence of specific pathologic features (304) (Table 15.3).

The onset of CD often is insidious, and it can even occur without gastrointestinal manifestations, especially in children. Suspicion should be raised by a characteristic history of chronic or nocturnal diarrhea, abdominal pain, and weight loss. Depending on disease location, diarrhea is often small in volume and associated with rectal urgency and tenesmus in patients with colonic disease or of large volume in cases of small bowel CD. Diarrhea in CD represents the combination of effects such as mucosal inflammation, impaired motility, bile salt catharsis due to severe inflammation, surgical resection of terminal ileum, malabsorption, bacterial overgrowth, and partial obstruction. Decreased bile salt absorption and deconjugation due to bacterial overgrowth in the setting of stricture formation may lead to steatorrhea. Patients with ileocolonic CD often have abdominal pain in the right lower quadrant that is associated with abdominal distention, satiety, nausea, and vomiting because of partial intermittent, ileal obstruction. Patients with CD limited to the colon commonly present with hematochezia, perianal complications, and extraintestinal complications involving skin, eyes, or joints (305). Anorexia, abdominal pain, nausea, malabsorption, and intestinal cytokine production result in weight loss. Low-grade fever is often associated with ileocecal disease; high fevers may occur with severe disease and suppurative complications. Occasionally, there may be a fulminant onset or toxic megacolon that cannot be distinguished from severe UC, but free intestinal perforation is less common than in UC because of thickening of bowel wall (176).

On physical examination, evidence of an abdominal mass caused by thickened and adherent bowel loops, an indurated mesentery, enlarged lymph nodes or an abscess, and abdominal tenderness, especially in the right lower quadrant in ileocecal disease, should be sought. Anemia, leukocytosis, and thrombocytosis are common laboratory findings. Megaloblastic anemia may ensue because of vitamin B_{12} and folic acid malabsorption. Hypocalcemia, hypomagnesemia, and hypoproteinemia indicate severe malabsorption.

Diffuse jejunoileitis is a variant of CD that can be complicated by multifocal stenoses, bacterial overgrowth, malabsorption, steatorrhea, and protein-losing enteropathy (306). Severe manifestations caused by gastric or duodenal ulceration and stricture-associated obstructions occur in only 1% to 5% of all patients with CD (307,308). However, focally enhanced gastritis has been found in 25% of patients with CD who had normal endoscopic findings (309). Rare esophageal CD is associated with dysphagia, odynophagia, chest pain, and dyspepsia.

TABLE 15.3. *Vienna classification of Crohn's disease*

Age at diagnosis[a]	A1: <40 years
	A2: ≥40 years
Location[b]	L1: Terminal ileum[c]
	L2: Colon[d]
	L3: Ileocolon[e]
	L4: Upper GI[f]
Behavior	B1: Nonstricturing nonpenetrating[g]
	B2: Stricturing[h]
	B3: Penetrating[i]

Further data to be collected:
Patient's name: _____ Date of birth: ___/___/___ Sex: female ☐ male ☐
Ethnicity: Caucasian ☐ black ☐ Asian ☐ other: _____ Jewish: yes ☐ no ☐ partly ☐
Family history of IBD: 1st degree relatives[j] ☐ other[k] ☐ none ☐
Extraintestinal manifestation: yes ☐ no ☐

[a] The age when diagnosis of Crohn's disease was first definitively established by radiology, endoscopy, pathology, or surgery.
[b] The maximum extent of disease involvement at any time before the first resection. Minimum involvement for a location is defined as any aphthous lesion or ulceration. Mucosal erythema and edema are insufficient. For classification at least both, a small bowel and a large bowel examination, are required. Use only one box.
[c] Disease limited to the terminal ileum (the lower third of the small bowel) with or without spillover into cecum.
[d] Any colonic location between cecum and rectum with no small bowel or upper gastrointestinal (GI) involvement
[e] Disease of the terminal ileum with or without spillover into cecum and any location between ascending colon and rectum.
[f] Any disease location proximal to the terminal ileum (excluding the mouth), regardless of additional involvement of the terminal ileum or colon
[g] Inflammatory disease that never has been complicated at any time in the course of disease
[h] Stricturing disease is defined as the occurrence of constant luminal narrowing demonstrated by radiologic, endoscopic, or surgical-pathologic methods with prestenotic dilatation or obstructive signs/symptoms without presence of penetrating disease at any time in the course of disease.
[i] Penetrating disease is defined as the occurrence of intraabdominal or perianal fistulas, inflammatory masses, or abscesses at any time in the course of disease. Perianal ulcers are also included. Excluded are postoperative intraabdominal complications and perianal skintags.
[j] Parents, siblings or children.
[k] Second or third degree; no spouses.

Abscesses complicating preexisting CD occur in 21% of patients because of occlusion of blind tracts (i.e., sinuses). Abscesses mostly arise spontaneously, sometimes postoperatively between intestinal loops, between intestine and peritoneum, in the mesentery, or in intrahepatic or intrasplenic sites, eventually extending into the iliopsoas and the retroperitoneum.

In CD, inflammation may extend through the serosa, leading to adherence to adjacent intraabdominal and pelvic structures. Fistulas may develop as pathologic communications between the luminal gastrointestinal tract and other bowel segments, organs, or skin, particularly in the perianal region, and usually occur proximal to a stricture. Enteroenteric fistulas are often small and incidental findings but are occasionally large enough to cause diarrhea, malabsorption, and weight loss. Pneumaturia, recurrent urinary tract infections, and even fecaluria are features of enterovesical fistulas. Rectovaginal fistulas are characterized by foul vaginal discharge or even gas or stool passage through the vagina.

Muscular hyperplasia, fibrosis, adhesions, and inflammatory infiltration result in intestinal obstruction, particularly of the small intestine, in up to 30% of patients. Crampy midabdominal pain and diarrhea that worsens after meals and improves with fasting are associated symptoms. In approximately 25% of patients, especially those with Crohn's colitis, perianal CD is present as involvement of the anal canal, complicated by perianal fissures, abscesses, or fistulas protruding to scrotum, vulva, or groin.

CD has a naturally remitting and recurring course. The placebo response rate varies from 8% to 44% of patients with active CD and approximately 30% of patients who achieved remission relapse within 1 year (310). An aggressive perforating CD characterized by abscesses or fistula formation with a short duration of disease before surgery has been differentiated from nonperforating CD, which takes an indolent clinical course associated with obstruction and bleeding (311). Upper respiratory tract and enteric intercurrent infections, cigarette smoking, the use of NSAIDs, the failure to comply with the maintenance regimen, and mesalamine sensitivity are factors exacerbating CD (312). The issue of stressful life events or psychologic predispositions initiating or exacerbating IBD remains controversial. Seasonal variations in onset and exacerbations for UC, but not CD, have been found and suggest the influence of environmental allergens (313).

Ulcerative Colitis

UC is a chronic disease characterized by mucosal inflammation limited to the colon. It involves the rectum and may extend proximally in a circumferential and uninterrupted pattern to involve parts or all of the large intestine. During the first attack, disease is limited to the rectum in approximately 30% of patients, extends to the hepatic flexure in about 40%, and involves the entire colon (extensive disease) in the remaining 30% (314). The classic feature of a continuous nature of colonic inflammation in UC has been blurred by the finding of skip areas, most often in the periappendiceal region in 75% of patients with left-sided UC (315). The hallmark clinical symptom of UC is bloody diarrhea with frequent bowel movements, often small in volume and associated with mucopus. In distal UC, blood is often present on the outside of the stool or may be passed without accompanying stool, whereas in cases of extensive disease, blood is typically mixed with the stool. Crampy lower abdominal pain, rectal urgency, and tenesmus are common. Weight loss, fever, and signs of toxicity are features of severe illness. Extensive disease, increased disease severity, and older age are parameters negatively influencing the outcome of the first UC attack (316).

UC is a spontaneously remitting disease with a placebo remission rate of approximately 10% and a placebo benefit rate of approximately 30% (317). However, in less than 8% of patients, no recurrence occurs after an initial acute attack during the next 10 years (318). The likelihood of relapse is not affected by colonic involvement or severity of the first attack, but an inverse correlation has been shown between age and recurrence (319). Intercurrent infection is a precipitating risk factor and seasonality to relapses from August to January has been observed (320,321).

Free perforation, massive hemorrhage, and toxic dilation are major complications of severe UC. Colonic perforation, most often in the sigmoid colon, occurs more often during the first attack of UC, because of the initial lack of muscularis hypertrophy and fibrosis, which develop over time and result in benign colonic strictures. Malignant strictures have to be identified. Toxic megacolon is the most serious complication, typically occurring in pancolitis and resulting from the extension of the inflammatory process beyond the submucosa to the muscularis, leading to colonic atony and distention. Antimotility agents, barium enema examinations, colonoscopy, and hypokalemia are risk factors for the development of this clinical setting. Patients present with fever, dehydration, tachycardia, hypotension, rebound tenderness over the colon, and reduced or absent bowel sounds. The patient may have anemia, leukocytosis, hypoalbuminemia, electrolyte disturbances, and metabolic acidosis.

Diagnostic Approach

No single standard has been established for the diagnosis of IBD, and the discrimination of UC and CD continues to be based on a combination of clinical, laboratory, endoscopic, histopathologic, and radiographic observations (322,323) (Table 15.4). The potential heterogeneity of the clinical presentations makes the diagnosis of CD, especially colonic disease, particularly difficult. In 10% to 20% of IBD cases, the definite allocation to UC or CD remains impossible by macroscopic and microscopic findings, and these are classified as indeterminate colitis (298,324). However, a precise diagnosis is required because the diseases differ in their natural course and complications, because of the opposing effect of cigarette smoking on disease severity (217,218), and the response to therapy. The high recurrence risk for CD within the ileal reservoir after ileal pouch–anal anastomosis underscores the necessity of a correct differential diagnosis (325).

Contrast radiography and endoscopy are often used in a complementary fashion as diagnostic tools. Upper or lower gastrointestinal tract endoscopy is used to assess disease location, extent, and severity, along with obtaining tissue for pathologic evaluation (326). Endoscopic biopsy can establish the diagnosis of IBD, occasionally distinguish between UC and CD, exclude acute self-limited colitis, or identify dysplasia or cancer as part of a surveillance examination (327). In 70% to 80% of colonoscopies, the intubation of the terminal ileum is successful, enabling the accurate endoscopic and histologic evaluation of this common site for CD. The accuracy of colonoscopy performed by experienced endoscopists in differentiating UC from CD is 85% to 90% (328,329). Contrast radiography is more effective in detecting colonic distensibility, strictures, and fistulas, but barium enema and colonoscopy are contraindicated in patients with moderate or severe UC because of the risk of toxic megacolon or colonic perforation. A combination of colonoscopy, if possible with ileoscopy of the terminal ileum, multiple colonic biopsies, and small bowel follow-through is appropriate to ascertain a diagnosis of CD or UC. The additional performance of gastroscopy with antral-corpeal biopsies can evaluate possible involvement of the upper gastrointestinal tract in CD and may help reduce uncertainties in deciding between CD and UC because of the frequent histologic finding of gastric minilesions in CD (309).

The earliest endoscopic finding of CD is the appearance of a red halo around a lymph follicle with microscopic, but not macroscopic, ulceration; it seems to precede visible aphthoid ulcers and suggests that these lesions originate from follicle-associated epithelium (330). Advanced endoscopic features of CD include aphthoid ulcerations, deep linear, serpiginous or fissure-like ulcers, strictures, fissures, and fistulas (Figs. 15.9 and 15.10). Linear ulceration with intervening areas of intact mucosa, often heaped because of inflammatory infiltration, produces a cobblestone appearance. CD involvement of the colon is usually segmental, and the rectum can be spared. Skip areas are often present between lesions, but occasionally Crohn's colitis may be diffuse, with rectal involvement. Benign strictures are usually concentric and smooth, whereas malignant strictures are more likely to be rigid, nodular, or eccentric. Generally, the endoscopic appearance of CD does not correlate with clinical disease activity and should not be

TABLE 15.4. *Differentiation of Crohn's disease and ulcerative colitis by anatomic, clinical, endoscopic, and histopathologic features*

Characteristic	Crohn's disease	Ulcerative colitis
Disease location		
Upper gastrointestinal tract	Occurs	Absent
Ileal involvem nt	70%, narrowed	15%, dilated, "backwash ileitis"
Rectal involvement	Occurs	95–100%
Perianal disease	Common	Absent
Postoperative recurrence	Common	Absent
Clinical presentation		
Influence of smoking on course of disease	Negative	Positive
Weight loss	Common	Occurs
Fever	Occurs	In severe cases
Abdominal pain	Common	Occurs
Tenesmus	Occurs	Common
Hematochezia	Occurs	Common
Right lower quadrant abdominal mass	Common	Absent
Complications		
Colonic distention	Rare	In severe cases
Stricture	Common	Rare
Abscess	Common	Rare
Fistulas	Common	Rare
Primary sclerosing cholangitis	Rare	Occurs
Pyoderma gangrenosum	Rare	Occurs
Erythema nodosum	Occurs	Rare
Right hydronephrosis	Occurs	Absent
Endoscopic features		
Mucosal involvement	Discontinuous	Continuous
Mucosal granularity, friability	Occurs	Common
Cobblestoning	Common	Absent
Ulcers	In normal mucosa	In abnormal mucosa
Correlation between clinical and endoscopic activity	Absent	Reasonable
Histopathologic features		
Epitheloid granuloma	Occurs	Absent
Crypt distortion, decreased crypt density	Occurs	Common
Lamina propria cellularity	Focal	Dense
Mucin depletion	Occurs	Common

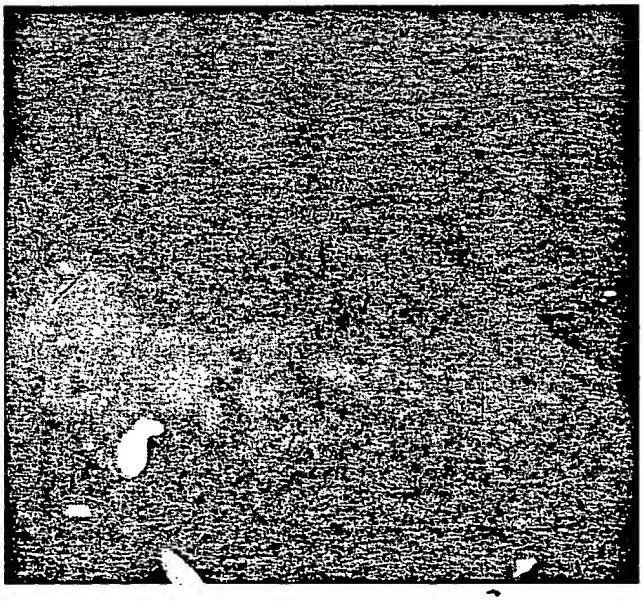

FIG. 15.9. Crohn's colitis with aphthous lesions among areas of macroscopically intact mucosa. (Courtesy of Schöfl)

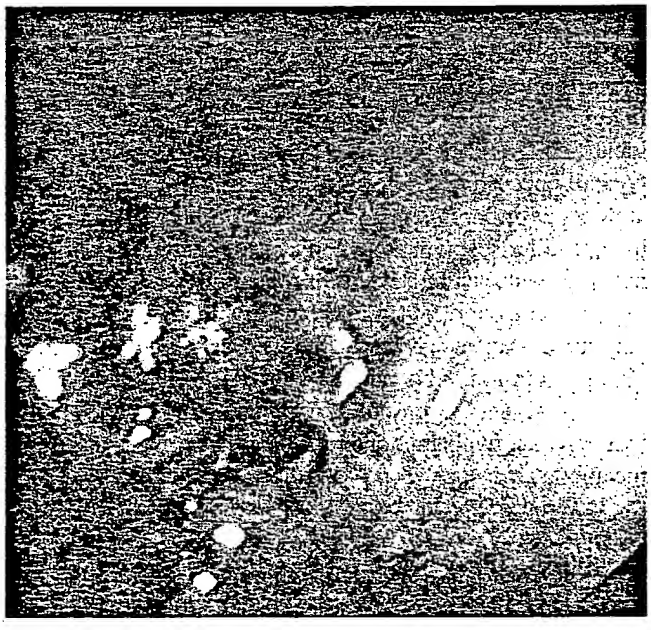

FIG. 15.10. Fistula formation in Crohn's disease of the terminal ileum. (Courtesy of Schöfl)

used to assess symptoms or response to therapy (331). However, colonoscopic evaluation of a surgical anastomosis can be used to predict the likelihood of clinical recurrence (332). Endoscopic findings described in gastroduodenal CD have included patchy or streaky mucosal reddening, edema, single or multiple nodularities, cobblestoning, erosions, and ulcers (333).

A complete examination of the small intestine is possible by two radiologic methods. For the small bowel follow-through assessment, patients drink a barium suspension, whereas for enteroclysis, a small bowel enema and air contrast are applied by a tube passed orally into the third portion of the duodenum or proximal jejunum. Data have shown that small bowel follow-through assessment is safer than enteroclysis for diagnosing the presence and extent of CD, is preferred by patients, and does not miss gastroduodenal disease (334), whereas previous studies described excellent sensitivity and specificity for enteroclysis (335). Linear ulcers located along the mesenteric border of the small intestine, displayed as a long, thin, linear barium collection opposite to an relatively uninvolved small bowel, are an important sign of CD (336). Detailed views of strictures, long segments of luminal narrowing, fistulas, sinus tracts, inflammatory masses, ulcerations, and a cobblestone appearance are important features of small bowel radiography (Figs. 15.11 and 15.12). Analogous lesions can be demonstrated in the colon by double-contrast radiography using a barium enema. For the evaluation of a stenosed bowel segment, contrast examination of the colon is particularly important. However, radiologic features of CD are not specific and may be observed in cases of bacterial infections such as *Yersinia* ileitis or tuberculosis (337).

In UC, endoscopy is the most accurate method of determining the extent of colonic disease. The lesions involve the distal rectum and proceed proximally in a continuous and circumferential pattern. Endoscopic findings in UC include a loss of the typical vascular pattern because of edema, erythema, granularity, and friability (Fig. 15.13). In severe UC, ulceration with a mucopurulent exudate and spontaneous bleeding may be apparent. Regenerative or residual inflammatory mucosa may be prominent as pseudopolyps (326). Endoscopic and histologic rectal sparing is unusual for UC and more often is associated with CD, unless topical steroids have been used in treatment. In cases of UC, clinical activity, endoscopic activity, and histology show a reasonable correlation, and the persistence of active inflammatory lesions determined by histologic examination in the setting of endoscopic remission predicts early relapse (338).

Plain radiographs in supine and upright positions should be performed for every patient with severe UC to detect colonic dilatation. A diameter of 5.5 cm or greater in the segment of

FIG. 15.12. Enteroclysis of ileal Crohn's disease with a cobblestone appearance. (Courtesy of E. Schober)

FIG. 15.11. Small bowel follow-through assessment of ileocolonic Crohn's disease with narrowing of the terminal ileum and fistula formation of the cecum. (Courtesy of E. Schober)

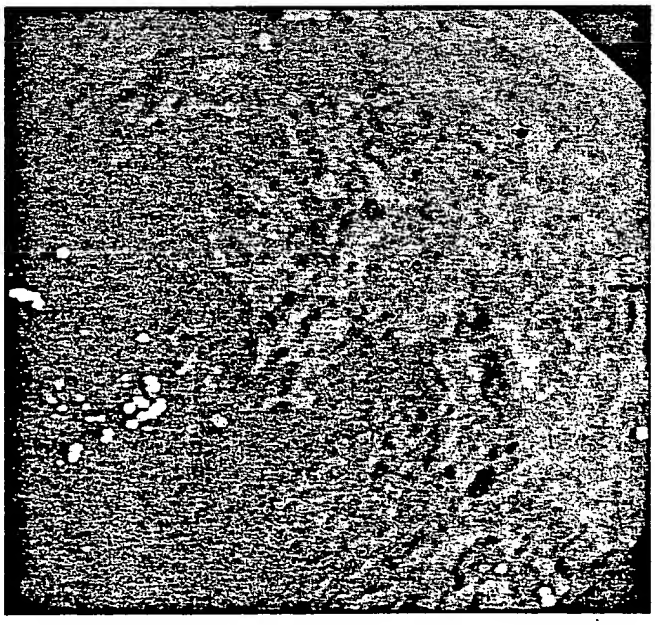

FIG. 15.13. Ulcerativ colitis with continuous and circumfer- ntial loss of the typical vascular pattern because of granularity and friability. (Courtesy of Schöfl)

the colon, which is highest in the abdominal cavity in the corresponding position, most commonly in the transverse colon, is an important sign in the diagnosis of toxic megacolon. Barium enema results in early UC may be normal or show limited distensibility, with a slightly irregular or granular mucosa. In more severe disease, coarse granularity, nodularity, pseudopolyps, and ulcerations are discernible. Severe proctitis results in enlargement of the presacral space. Backwash ileitis can be observed in 15% to 20% of patients with pancolitis. In long-standing UC, the colon is shortened and has a tubular appearance because of loss of haustral markings. Suspicion of colon cancer should be raised in the cases of masses, flattened or rigid areas, and strictures.

Transabdominal bowel sonography (TABS) is a safe and useful diagnostic method to obtain information about transmural changes of the small and large bowel, excluding the rectosigmoid, in patients with IBD (339). TABS is an accurate method for the detection of intestinal complications in CD, such as strictures, intraabdominal fistulas, or abscesses (340). Computed tomography or magnetic resonance imaging are also appropriate to discriminate intraabdominal masses and abscesses (341). Enteroclysis spiral computed tomography (CT) is an accurate method for the detection of mucosal and extramucosal abnormalities in patients with CD (342). Radiolabeled leukocyte scans can assist assessment of localization, extent, and degree of active inflammation in IBD (343).

An increased prevalence of pANCAs has been described for the sera of patients with UC (344). However, the significant proportion of pANCA-negative UC patients and the identification of a small subgroup of CD patients with pANCA positivity as a specific clinical phenotype with features of UC limits the clinical utility of this serologic marker for differentiation of the diseases (345). The combination of ASCAs with ANCAs may become a useful diagnostic tool for differentiating UC from CD.

TABLE 15.5. *Evaluation of inflammatory bowel disease severity*

Crohn's disease
Mild to moderate Crohn's disease applies to ambulatory patients able to tolerate oral alimentation without manifestations of dehydration, toxicity (high fevers, rigors), abdominal tenderness, painful mass, or obstruction.
Moderate to severe Crohn's disease applies to patients who have failed to treatment for mild to moderate disease or those with prominent symptoms of fever, significant weight loss (more than 10%), abdominal pain and tenderness (without rebound), intermittent nausea or vomiting (without obstructive findings), or significant anemia.
Severe or fulminant Crohn's disease refers to patients with persisting symptoms despite the introduction of steroids on an outpatient basis or individuals presenting with high fever, persistent vomiting, evidence of intestinal obstruction, rebound tenderness, cachexia, or evidence of an abscess.
Remission refers to patients who are asymptomatic or without inflammatory sequelae and includes patients who have responded to acute medical intervention or undergone surgical resections without gross residual disease. Patients requiring systemic steroids are usually not considered to be in remission.
Ulcerative colitis
Mild disease applies to patients who have four stools daily or less, without or only with small amounts of blood, no systemic signs of toxicity, and a normal erythrocyte sedimentation rate.
Moderate disease applies to patients who have more than four stools daily but have minimal signs of toxicity.
Severe disease is defined as six or more bloody stools daily and evidence of toxicity as demonstrated by fever, tachycardia, anemia, or an elevated erythrocyte sedimentation rate to 30 mm/hour or more.

[a] The criteria for Crohn's disease (CD) are formulated according to the working definitions of the American College of Gastroenterology for the development of guidelines in the management of CD. The three-degree evaluation of ulcerative colitis is adapted from the criteria of Truelove and Witts (347).

Disease Activity

Assessment of disease activity is essential for planning management of IBD, for evaluating treatment, and for determining prognosis (346). Clinical and endoscopic activity indices are available for UC and CD, but they are largely used in clinical and drug studies.

As initially proposed by Truelove and Witts, a three-degree evaluation of clinical severity in UC is usually performed (347) (Table 15.5). During a first attack, UC is mild in more than 50% of patients (most often in patients with distal disease), moderate in approximately 25%, and severe in approximately 20%. Other clinical indices such as the severe clinical activity index proposed by Rachmilewitz and more quantitative activity scores (including endoscopy) are available (348,349). Overall, frequency and the amount of blood in stools, abdominal pain, incontinence, and signs of toxicity are important symptoms to describe the clinical activity in UC. Combination with the description of possible extraintestinal manifestations, abdominal tenderness, and endoscopic findings permits a good evaluation of disease severity.

Defining disease activity in CD is complicated by the heterogeneous patterns of disease location and complications and by the lack of a standard indicator of clinical disease. The discrepancy between histologic investigation, endoscopic appearance, and symptoms or clinical indices is often profound (331,350,351). Disease severity may be established on clinical parameters differentiating localized inflammatory, obstructive, or fistulizing processes; systemic and extraintestinal manifestations; and the global impact of the disease on the individual's quality of life as outlined in the working definitions of the American College of Gastroenterology for the development of guidelines in the management of CD (352) (Table 15.5).

Different indices have been developed to rate disease activity in CD, but the most commonly used measurement is the CD activity index (CDAI) (353). The CDAI was developed

to objectively assess response to therapy by incorporating variables that have been identified by multiple regression analysis to best predict disease activity. The included items are the number of liquid or very soft stools, abdominal pain, general well-being, extraintestinal manifestations, abdominal mass, use of antidiarrheal drugs, hematocrit, and body weight. The total score is obtained by summing the products of the grade of each variable and its weighting factor, and the total ranges from 0 to 600. Scores below 150 indicate remission, and scores above 450 signify severe illness. The considerable impact of subjective elements on the CDAI and its impairment by complications such as stenosis or functional disorders after bowel resection, the need for registration of symptoms for 7 days, and the inclusion of the hematocrit (known to be a poor measure of disease activity) has lead to the criticism that the CDAI is a measure of illness rather than of inflammatory activity.

An index including, apart from stool consistency, only objective variables and biochemical values was developed by Van Hees et al. (354). Disease-specific instruments to measure quality of life factors in IBD have been designed (355). The 32-item IBD questionnaire (IBDQ) measures four quality of life dimensions: bowel, systemic, social, and emotional factors. The only validated endoscopic CD activity index is the CD endoscopic index of severity (CDEIS), assessing the percentage of CD-affected mucosal surface in five segments of the intestine: rectum, sigmoid and left colon, transverse colon, right colon, and ileum. However, no relation between clinical activity and endoscopic severity exits (356). An endoscopic score to evaluate the severity of postoperative recurrence of CD was introduced by Rutgeerts et al. (332).

Acute-phase reactants (e.g., orosomucoid, C-reactive protein), erythrocyte sedimentation rate, platelet count, and serum albumin have been used as nonspecific parameters to monitor inflammatory activity in IBD (357,358). Based on the increasing notions of the pathophysiologic mechanisms engaged in IBD, other markers of inflammation have been tested to assess disease activity such as cytokines (e.g., IL-6, IL-8) and soluble cytokine receptors (e.g., IL-2R) (264). Fecal excretion of lactoferrin and increased gut permeability have also been considered for evaluation of disease activity in patients with IBD (359,360).

Recurrence of Crohn's Disease After Resection

After curative resection with ileocolonic anastomosis, CD recurs within months in the neoterminal ileum, beginning as aphthous lesions (361,332). One year after surgery, endoscopic and radiologic recurrence rates of 27% to 75% have been described (362). Patients with severe endoscopic or radiologic findings become symptomatic within 1 to 3 years. Recurrence is triggered by the fecal stream and luminal bacteria (363). Adverse risk factors for early recurrence are aggressive disease, high preoperative inflammatory disease activity, multiple bowel resections, and smoking (364,365).

Extraintestinal Manifestations

Extraintestinal manifestations affect 25% to 30% of patients with IBD, with joint involvement occurring most frequently, followed by skin lesions, PSC, and ocular manifestations (366,367). The pathogenesis of the extraintestinal disorders is largely unknown, but some, such as erythema nodosum and peripheral arthritis, appear directly related to the severity of and medical response to the colonic inflammation, whereas others, such as PSC, ankylosing spondylitis, sacroiliitis and sometimes pyoderma gangrenosum, may progress independently of the colitis activity. The disorders may manifest before, at the same time, or after the onset of colonic symptoms, as well as after colectomy (368).

Peripheral arthritis, occurring in 25% of cases, is the most common extraintestinal manifestation of IBD and is characterized by asymmetric pain or painful swelling involving the knees, hips, ankles, elbows, and wrists without bony destruction or evidence of other rheumatic diseases. Enteropathic peripheral arthropathy without axial involvement can be subdivided into a pauciarticular, large joint arthropathy that is most commonly associated with relapsing IBD and a bilateral, symmetric polyarthopathy that is associated with persistent symptoms (369). The migration of activated, intestinal lymphoblasts to synovial tissue by means of the vascular adhesion protein-1 of endothelial cells may be a mechanism of reactive arthritis in IBD (370). Effective anticolitic therapy usually results in improvement of peripheral arthritis. NSAIDs may relief arthritic pain but carry the risk of triggering a relapse of IBD.

Ankylosing spondylitis with or without sacroiliitis, mostly associated with positivity for HLA-B27, affects 2% to 6% of patients with UC. Patients present with low back pain, morning stiffness, and a stooped posture. The treatment comprises physical rehabilitation, sulfasalazine, and NSAIDs (371).

Pyoderma gangrenosum, more frequently associated with UC, and erythema nodosum, more often occurring in CD, are the most striking skin manifestations of IBD and are seen in 15% of patients. Erythema nodosum is characterized by raised, tender nodules, usually occurring over the anterior surface of the tibia. Pyoderma gangrenosum manifests as an expanding, often large, and deep ulcer with a necrotic base on the leg. Whereas erythema nodosum improves with effective therapy of colitis, no predictably successive therapy exists for pyoderma gangrenosum. Various medications, including local, oral, or intravenous corticosteroids, sulphasalazine, azathioprine, cyclosporine, or dapsone, have been tested empirically.

Ocular manifestations occur in about 5% of patients with IBD. In UC, the serious condition of anterior uveitis, presenting as eye pain, photophobia, blurred vision, and conjunctival injection, is prominent in 0.5% to 3% of patients. It requires an urgent diagnosis by slit-lamp examination and therapy with topical corticosteroids. Scleritis and episcleritis presenting as scleral injection and burning are milder ocular complications that are more frequently seen in CD.

PSC is the major hepatic disease in IBD, occurring in 3% to 5% of patients with UC and in a smaller percentage of the patients with CD. Approximately 75% of patients with PSC have associated IBD. The manifestations of the disease may vary from asymptomatic intrahepatic limitations to progressive intrahepatic and extrahepatic periductal fibrosis accompanied by cholestasis, secondary bacterial cholangitis, cholangiocarcinoma, and liver cirrhosis with hepatic insufficiency and portal hypertension (372). The clinical course of PSC does not parallel activity of the colitis, and the disease may progress or occur after colectomy. Controversy exists regarding PSC as a risk factor for the development of right-sided colorectal dysplasia or cancer in patients with UC (373,374). The presence of PSC has important implications for surgical treatment of colitis, because proctocolectomy with ileostomy may lead to peristomeal varices, and ileal pouch–anal anastomosis is associated with a higher frequency of pouchitis. Orthotopic liver transplantation is the only life-sustaining and potentially curative therapy for PSC. Ursodeoxycholic acid has improved biochemical liver function test results but failed to prolong survival without liver transplantation in cases of advanced disease. The effect of ursodeoxycholic acid on the course of early PSC cannot be excluded (375). Up to 30% of patients with IBD have asymptomatic elevations of the liver functions without PSC (376). Other hepatic complications of IBD include fatty liver because of weight loss and malnutrition.

Thromboembolic complications occur in 1% to 39% of patients with IBD and are associated with a high mortality rate (319,377). Activation of blood coagulation, with increased serum levels of factors V and VIII and fibrinogen, as well as decreased levels of protein C, protein S, antithrombin III, and factor XIII, has been recognized, but no consistent abnormalities have been observed in all patients. Platelet dysfunction and thrombocytosis may be related to the risk of thromboembolism (378). A thrombotic pathogenesis of IBD with mesenteric microvascular occlusion has been suggested. The epidemiologic finding that inherited disorders of coagulation appear to protect against IBD is consistent with this hypothesis (379). Factor XIII substitutions and heparin are beneficial in patients with active UC, but this approach is not routinely practiced.

Clinically significant renal or urologic complications occur in 10% to 15% of patients with IBD and may be related to complications of the intestinal disease process (e.g., macalculous hydronephrosis, fistula formation, abscess), metabolic or inflammatory consequences of the disease (e.g., urolithiasis, amyloidosis), medication side effects (e.g., renal tubular damage from 5-ASA), or interpreted as extraintestinal manifestation of IBD (e.g., renal tubular and glomerular nephropathies) (380–382). Pancreatitis, pancreatic dysfunction, and focal white matter lesions in the brain have been associated with IBD (383,384). Rare pulmonary complications, especially in patients with UC, include pulmonary infiltrates with eosinophilia, bronchiolitis obliterans, pulmonary nodules, and serositis. However, pulmonary function abnormalities have been observed in 55% of patients with UC but are not related to a family history of pulmonary disease or to current or previous smoking status (385).

Osteopenia, osteoporosis, and osteomalacia are frequent metabolic bone diseases in IBD, occurring in more than 50% of cases. Dual-energy x-ray absorptiometry has provided a practical tool for diagnosis. Several mechanisms may be involved in IBD-associated bone disease. Vitamin D and calcium deficiency due to malabsorption and reduced intake may activate bone turnover. Treatment with corticosteroids may exert catabolic effects on the bone, and patients with a total lifetime steroid dosage of more than 5 to 10 g of prednisone or equivalent doses should be considered at increased risk for osteoporosis. During acute phases of IBD, immobilization may predispose the patient to high-turnover bone disease. The generation of cytokines such as IL-1 and IL-6 in the inflamed intestinal mucosa may directly induce bone degradation (386).

CD may be complicated by metabolic disorders related to small bowel malabsorption such as nephrolithiasis (from hyperoxaluria after intestinal resection), cholelithiasis, or anemia (from iron deficiency). Anemia in more than 80% of patients with IBD is caused by iron deficiency due to chronic intestinal blood loss and folate or vitamin B_{12} malabsorption. Inadequate erythropoietin production based on the intestinal overproduction of antagonizing proinflammatory cytokines may also contribute to this symptom (387). In some cases, hemolysis or myelosuppression occurs. Anemia occurs in about one third of patients with CD and, in its severe form, as defined as a hemoglobin concentration of 10.5 g/dL, in approximately 15% of cases.

Differential Diagnosis

IBD must be differentiated from other specific or idiopathic IBDs that may mimic or sometimes complicate the clinical course. Stools should be examined for the presence of enteric pathogens, ova and parasites, and *Clostridium difficile* toxin. A serologic exclusion of amebic infection must be performed (176) (Table 15.6).

Especially in patients at risk for AIDS, intestinal tuberculosis should be differentiated from CD. Tuberculosis may involve the entire gastrointestinal tract, but the ileocecal region is the most common site of infection that may lead to the development of inflammatory mass, fistulization, bowel narrowing, and lymph node enlargement. Fistula and intestinal stenosis may also result from chronic fungal infections such as actinomycosis or blastomycosis. *Yersinia* ileitis may resemble Crohn's ileitis, and various bacterial pathogens such as *Salmonella, Shigella, Campylobacter jejuni,* or *E. coli* O157:H7 can cause an UC-like illness. Lymphogranuloma, infection with *Chlamydia*, herpesvirus, and cytomegalovirus and syphilis or gonorrhea may lead to proctitis in homosexual men. In HIV-infected patients, opportunistic infections such as *Mycobacterium avium* complex, *Cryptosporidium, Microsporidium,* and *Isospora* may cause diarrhea and abdominal pain.

TABLE 15.6. *Differential diagnosis of inflammatory bowel disease*

Salmonella, Shigella
E. coli 0157:H7
Campylobacter jejuni, Yersinia enterocolitica
Tuberculosis
Amebiasis, giardiasis, *Plesiomonas, Aeromonas*
Gonorrhea, syphilis, lymphogranuloma venereum
Chlamydia
Herpes simplex, cytomegalovirus
Cryptosporidium, Microsporidium, or *Isospora*
Clostridium difficile
Actinomycosis, blastomycosis
Diverticulitis
Radiation enteritis
Ischemic bowel disease
Diversion colitis
Solitary rectal ulcer syndrome
Cathartic colon
Irritable bowel syndrome
Drug-induced colitis
Collagenous and lymphocytic colitis
Carcinoma, lymphoma
Carcinoid syndrome
Eosinophilic enteritis
Vasculitis
Behçet's syndrome

Diverticulitis may be confused clinically and radiographically with CD, but endoscopy is helpful, because in CD, the abnormality of mucosa is more evident. Radiation enteritis may appear many years after therapy is completed and can be complicated by stricturing or fistulization. Apart from mucosal granularity, friability, and ulcerations, telangiectases are often observed in late radiation colitis. The spectrum of ischemic bowel disease may encompass early edematous lesions to gangrenous bowel. Hypercoaguable states in the setting of neoplastic or hematologic disorders, severe cardiac or peripheral vascular disease, and vasculitides can predispose to ischemic colitis, which typically centers on the splenic flexure and often spares the rich vascularly supplied rectum. Diversion colitis may develop in a segment of the colon that has been surgically bypassed and results from depriving the intestinal epithelium of the metabolic fuel (e.g., glutamine, short-chain fatty acids) derived from the intestinal lumen. As a consequence, the mucosal barrier is compromised and penetrated by luminal proinflammatory mediators.

Solitary rectal ulcer syndrome, cathartic colon, irritable bowel syndrome, collagenous and lymphocytic colitis, and colitis induced by drugs such as NSAIDs, gold, estrogen, and allopurinol occasionally mimic IBD.

Fertility and Pregnancy in Inflammatory Bowel Disease

Women with IBD are often at their peak of reproductive life and therefore likely to undergo pregnancy. Fertility of women with IBD appears to be normal, but a significant proportion of patients avoid sexual activity and pregnancy because of dyspareunia and emotional or cosmetic reasons. The incidence of spontaneous abortions, stillbirths, prematurity, and congenital abnormalities is comparable to that for a normal population of patients with inactive disease, but in the case of active disease, increased frequencies have been described (388). Physicians' efforts should be directed at inducing remission before women become pregnant. Male infertility due to sulfasalazine-induced, reversible oligospermia, decreased motility, and abnormal sperm forms may contribute to decreased numbers of pregnancies in IBD couples.

Pregnancy does not increase the risk of relapse for a patient with IBD when the disease is quiescent at the time of conception. However, for patients with active IBD at the time of conception, approximately one third will improve, one third will worsen, and one third will remain the same. New-onset IBD during pregnancy is no more severe than that in nonpregnant women and occurs most often during the first trimester or in the postpartum period. Sigmoidoscopy and biopsy are not contraindicated to diagnose and evaluate the course of IBD during pregnancy. Colonoscopy and radiologic examinations should be avoided, especially in the first trimester. Steroids and sulphasalazine should be used in pregnancy in the same way as they are in nonpregnant women. The new salicylates and azathioprine are most probably safe during pregnancy. Cyclosporine may be chosen as an alternative to surgery in treating steroid-refractory UC (389).

Inflammatory Bowel Disease in Children

About 2% of all patients with IBD present before the age of 10 years, but 30% develop symptoms between the ages of 10 and 19 years. The classic gastrointestinal symptoms of bloody diarrhea, abdominal pain, and weight loss, as well as distribution of bowel involvement and response to therapy, are similar in children and adults. However, growth failure is a unique feature in pediatric IBD, particularly those with CD. Absolute height deficits are reported in 10% to 40% of these patients, and height velocities may be reduced in 88%. Growth retardation can be the first symptom of IBD and is often already present before other symptoms become apparent. The inflammatory process, accompanied by an increased caloric requirement, malabsorption, and malnutrition, plays a more important role in the occurrence of growth faltering than steroid treatment (390). Growth failure is an important marker of disease activity and the success of therapy (391). Enteral nutrition with an elemental or semi-elemental liquid diet is used as an alternative to corticosteroids in treating pediatric IBD, and energy and protein intake can be increased to 150% of recommended daily allowances for height and age (392). Depression and delayed puberty are also signs of ongoing IBD in children and adolescents (393).

Therapy

Treatments for IBD with sulfasalazine or 5-aminosalicylic acid (5-ASA), glucocorticosteroids, and immunosuppressants

are based on antiinflammatory or immune-modulating mechanisms. Progress in the understanding of the pathogenetic mechanisms of IBD has produced promising therapy strategies. However, a curative conservative management is still lacking because of the obscurity of the cause of the diseases.

Before establishment of therapy, the differential diagnosis must consider CD and UC, the extent and location of disease, and disease activity, and complications must be identified. The therapeutic recommendations are formulated according to the guidelines for the management of CD and UC in adults developed under the auspices of the American College of Gastroenterology and its Practice Parameters Committee (352,394).

Management of Crohn's Disease

CD is neither medically nor surgically curable. Therapeutic approaches aim to maintain clinical remission, optimize quality of life, and minimize short- and long-term toxicity. The medical guidelines presented are primarily organized according to disease severity with modifications, where applicable according to disease location and are divided into acute and maintenance phases (395) (Table 15.7).

Mild to Moderate Active Crohn's Disease

Sulfasalazine and 5-ASA represented major advances in the treatment of IBD. In sulfasalazine, the antiinflammatory 5-ASA is attached by an azobond to the antibiotic sulfapyridine, which is split and released by colonic bacteria. The demonstration that side effects of sulfasalazine are mostly attributed to the sulfonamide component and that 5-ASA is the active moiety of this compound led to the development of new oral 5-ASA formulations. In some of the new drugs, the sulfapyridine has been replaced, and 5-ASA has been linked by a nitrogen bridge to another carrier, such as another 5-ASA in olsalazine or 4-amino-benzoyl-β-alanine in balsalazide. In oral delayed-release preparations of 5-ASA (mesalamine), 5-ASA is not linked to a carrier but is coated with a semipermeable ethyl cellulose membrane or with acrylic resin, which retards release of the active molecule, especially in the colon. The therapeutic activity of sulfasalazine and 5-ASA in IBD is attributed to several mechanisms, including inhibition of platelet-activating factor, of the 5-lipoxygenase products prostaglandin E_2 and thromboxane B_2, and of interleukin-1 and TNF-α release. A reactive oxygen metabolite scavenging function, a suppression of antibody secretion, and an inhibition of the impaired epithelial barrier function induced by IFN-γ have been described (257,396–398). Intolerance to the sulfapyridine moiety of sulfasalazine is not uncommon and may result in nausea, vomiting, dyspepsia, anorexia, and headache. More severe, but less common adverse reactions to aminosalicylates include allergic reactions, diarrhea, pancreatitis, hepatoxicity, eosinophilia, cytopenia, hemolytic or megaloblastic anemia, coagulation disorders, renal disorders (e.g., renal tubular dysfunction, interstitial nephritis), pericarditis, myocarditis, lung disorders, rheumatologic and neurologic disorders, and male hypofertility.

Sulfasalazine at daily divided doses of 3 to 6 g is more effective than placebo in the control of active ileocolonic or colonic CD, but it is not consistently effective in patients with disease limited to the small intestine alone. Treatment with different oral 5-ASA formulations at doses of 3.2 to 4.8 g daily in divided doses may be of similar or even superior efficacy compared with sulfasalazine (399,400). Response to initial therapy should be evaluated after several weeks. Active therapy should be continued to the point of symptomatic remission, and patients in remission should be entered into a maintenance program. Symptoms of gastroduodenal disease have been reported to respond to acid-reduction therapy with omeprazole (401). Jejunoileitis is often complicated by small bowel bacterial overgrowth that responds to antibiotics (402).

TABLE 15.7. *Treatment of Crohn's disease*

Severity	Treatment
Mild to moderate	Oral sulfasalazine or 5-ASA
Moderate to severe	Oral steroids or oral budesonide (elemental or nonelemental dietary therapy; azathioprine, 6-mercaptopurine)
Severe or fulminant	Oral or intravenous steroids; surgery
Steroid-dependent and steroid-refractory disease	Azathioprine and 6-mercaptopurine; anti-TNF-α antibody
Perianal disease	Oral metronidazole or ciprofloxacin; oral steroids (addition of azathioprine and 6-mercaptopurine); intravenous cyclosporine (?); surgery
Maintenance	Azathioprine and 6-mercaptopurine, oral 5-ASA (?); anti-TNF-α antibody (?)

5-ASA, 5-aminosalicylic acid; TNF, tumor necrosis factor.

Moderate to Severe Active Crohn's Disease

After exclusion of infection or abscess, patients with persistent symptoms under treatment with oral sulfasalazine or 5-ASA and patients with moderate-severe clinical presentation should be treated with glucocorticosteroids (equivalent to 0.5 to 0.75 mg/kg of prednisone), which are the most effective therapy for active CD and are superior to sulfasalazine (403–405). Generally, doses are tapered by 5 to 10 mg/week until 20 mg and by 2.5 to 5 mg/week from 20 mg to discontinuation. Clinical remission induced by oral steroids is not paralleled by major improvement of endoscopic lesions, pointing to the uselessness of endoscopic monitoring of steroid therapy (406). Prednisone and prednisolone are effective in the induction of remission but have many short-term adverse effects. Nearly one half of the patients treated

acutely with steroids become steroid dependent or steroid resistant after the acute course, causing adrenal suppression and long-term toxicity, including osteoporosis, aseptic necrosis, cataracts, or hypertension (407).

Oral application formulations of budesonide, a semisynthetic topical active glucocorticoid with low systemic activity because of its rapid hepatic inactivation, have been developed as substitutes for conventional glucocorticosteroids. In a dosage of 9 mg/day, the controlled ileal release form of budesonide (Entocort) is similarly effective to induce remission in active ileal and right ileocolonic CD compared with systemic glucocorticoids and superior to sustained release mesalamine but less toxic (408–410). In smaller studies, efficacy was also shown for an oral, pH-dependent–release budesonide (Budenofalk) in treating active CD (411).

Glucocorticoids exert extensive antiinflammatory and immunosuppressive actions by inhibiting the expression and action of most cytokines and other mediators by multiple mechanisms. The activated glucocorticoid-receptor complex can bind and inactivate proinflammatory transcription factors (e.g., AP-1, NF-κB), upregulate the expression of cytokine inhibitory proteins (e.g. I-κB), and reduce the half-life time of cytokine mRNAs (412,413).

The application of total elemental diets and liquid polymeric diets implemented by oral administration or by nasogastric (nasoenteric) tubes by reducing antigenic stimulation with luminal contents has been investigated in CD. Although placebo-controlled trials of total enteral nutrition in CD have not been conducted, this therapy does appear to have a therapeutic benefit (414). However, meta-analysis confirms that steroids are more effective than elemental or nonelemental dietary therapy (415,416). Nutritional therapy is indicated in children with growth retardation.

Patients with more extensive disease and without indications for surgery should be treated concurrently with azathioprine or 6-mercaptopurine, which can enhance the short-term response to steroids (417).

Severe or Fulminant Crohn's Disease

Patients with severe or fulminant disease should be hospitalized. Intraabdominal abscesses should be evaluated by ultrasound or CT and need percutaneous or surgical drainage. Surgical consultation is also warranted for patients with obstruction. In patients with inflammatory disease who failed to respond to oral steroids, parenteral corticosteroids equivalent to 40 to 60 mg of prednisone are administered in divided doses or as a continuous infusion. After induction of remission, parental steroid therapy can be gradually transferred to an oral regimen. Nutritional support by elemental feeding or parenteral hyperalimentation is indicated for patients unable to tolerate an oral diet for more than 5 to 7 days. Patients with evidence of obstruction because of inflammatory narrowing, fibrotic stricture, or an adhesive process should be treated with bowel rest (418). Differentiation of the obstructive cause is based on prior radiologic or endoscopic studies, the clinical course, and laboratory signs of inflammation. Adhesive obstructions typically respond to nasogastric suction. Fibrostenotic disease may respond initially to bowel rest and steroids, but obstructive symptoms often recur with steroid tapering.

Dehydrated patients are resuscitated with fluid and electrolytes. Blood transfusions are indicated in the setting of active hemorrhage or symptomatic anemia. Broad-spectrum antibiotic therapy is indicated in the presence of inflammatory mass.

Perianal Disease

Nonsuppurative perianal complications of CD have a good response to metronidazole alone or in combination with ciprofloxacin (419,420). Perianal fistulas need continuous metronidazole treatment to minimize recurrent drainage, which is limited by the development of neurologic side effects (421,422). There is increasing evidence that immunosuppressive agents and intravenous cyclosporine are beneficial in treating perianal fistulas (423).

Maintenance Therapy in Crohn's Disease

Corticosteroids should not be used as long-term agents to prevent relapse of CD because of their side effects. Trials with controlled ileal release budesonide also have been disappointing regarding a consistent maintenance benefit (424).

The immunomodulating purine analogues 6-mercaptopurine (6-MP) and its prodrug azathioprine (AZA) are the only medications that provide maintenance benefits after a latent period of 2 to 6 months, allowing reduction in steroid doses in patients with steroid dependence (425,426). Doses varied in the different trials, but AZA at 2.5 mg/kg and 6-MP at 1.5 mg/kg have been effective and generally well tolerated. Mucosal healing was demonstrated with AZA in patients with steroid-refractory Crohn's ileitis recurring after operation (427). The complete blood count must be monitored carefully early in the course of treatment and long term at a minimum of every 3 months because of the risk of initial or delayed leukopenia (429). It remains to be determined whether leukopenia is necessary to induce an optimal response (430). Pancreatitis occurs in 4% to 15% of patients; fever, rash, nausea, diarrhea, and opportunistic infections, especially in combination with steroids, have also been observed. The risk of malignancy is not increased relative to the general population (431). Teratogenesis has not been observed in humans. A concomitant therapy with allopurinol should be avoided because of competitive inhibition of metabolic pathways. The benefit of continuing therapy with 6-MP and AZA over 4 to 5 years has yet to be defined (432). In addition to the steroid-sparing effect of AZA, this drug has healed fistulas in two thirds of patients. Promising results with 6-MP have been presented concerning the prevention of clinical, endoscopic, and radiographic postoperative relapse (433).

Results from a trial of intramuscular injection of the folate analogue methotrexate (25 mg/week) demonstrated efficacy in discontinuing or tapering prednisone in 39% of active steroid-dependent patients, but further controlled studies are needed to clarify its effects for long-term remission (434,435). Potential side effects include mild nausea, rash, leukopenia, thrombocytopenia, allergic pneumonitis, and hepatic fibrosis. The drug should not be used during pregnancy (436).

Cyclosporine, an immunosuppressant undecapeptide derived from a soil fungus, is used in organ transplantation for its selective depression of helper T-cell function and inhibition of IL-2 release. Oral cyclosporine is not indicated for therapy of active CD (437,438). However, closure of a fistula may become an indication for continuous intravenous cyclosporine (439).

Oral tacrolismus appears to be effective as a rapidly bridging therapy to long-term treatment with AZA or 6-MP, but much more study is needed to determine the role of tacrolismus in IBD (440).

The efficacy of mesalamine for the maintenance of remission in patients with CD has been investigated in several controlled trials, but the benefit is controversial. Doses above 3 g/day may be considered to reduce the likelihood of recurrence after surgical resection, but the overall benefit is only borderline. The issue of mesalamine in the maintenance treatment of CD in a postmedical setting is less clear. By subgroup analysis, it appears that women, patients with ileal disease, and patients with prolonged disease duration have some benefit from mesalamine, but its effect seems to diminish as time passes. Long-term steroid-sparing activity of mesalamine has not been documented (441–446). Mesalamine is safe for the management of IBD during pregnancy. The exposure to mesalamine during pregnancy increased preterm deliveries and decreased birth weight but did not result in major or minor malformations (447,448).

Management of Ulcerative Colitis

The goals of treatment in UC are induction and maintenance of clinical and mucosal remission (449). The endoscopically delineation of the proximal margin of inflammation determines the therapeutic management in UC. A distal inflammation, limited to below the splenic flexure, is within the reach of topical therapy, whereas extensive disease extending proximal to the splenic flexure requires systemic medication (Table 15.8).

Mild to Moderate Distal Ulcerative Colitis

Topical mesalamine and oral therapy with aminosalicylates are equally effective in achieving and maintaining remission in mild to moderate distal colitis (450–453). A topical approach should always be the first choice in treating proctitis or distal colitis. Advantages of topical therapy include a quicker response time and a less frequent dosing schedule. Topical vehicles applied as suppositories reach about 10 cm proximally, foam reaches 15 to 20 cm, and enemas reach up to the splenic flexure. 5-ASA suppositories (500 mg twice daily) are effective in the treatment and maintenance of proctitis. In patients with left-sided colitis 5-ASA enemas (2 to 4 g daily) are the first choice in inducing and maintaining remission (454). Conventional topical corticosteroids (100-mg hydrocortisone enema, 10% cortisone foam) and rectal budesonide (2-mg budsonide enema) are clearly superior to placebo but less effective than rectal 5-ASA for inducing remission of symptoms, endoscopy, and histology and have not proven effective in maintaining remission (455). The addition of oral 5-ASA to topical therapy in patients with distal UC may be more effective than either therapy alone (456).

Refractory distal colitis is defined as active distal inflammation unresponsive within 4 to 6 weeks to topical treatment with 5-ASA or corticosteroids with oral salicylates or sulfasalazine (457). Dosage increases and a drug switch of 5-ASA to corticosteroids and *vice versa* are logical. Topical administration of cyclosporine, nicotine tartrate, lidocaine, or other anesthetics are possible but need confirmation. Oral or intravenous application of steroids may become necessary.

Mild to Moderate Extensive Ulcerative Colitis

Patients with mild to moderate extensive colitis should begin therapy with oral sulfasalazine in daily doses titrated up to 4 to 6 g/day or an alternative aminosalicylate in doses up to 4.8 g/day (458). Approximately 80% of patients intolerant to sulfasalazine tolerate mesalamine and olsalazine (459). Data

TABLE 15.8. *Treatment of ulcerative colitis*

Severity	Distal disease	Extensive disease
Mild	Topical 5-ASA or steroids	Oral sulfasalazine or 5-ASA
Moderate	Topical 5-ASA or steroids plus oral sulfasalazine or 5-ASA	Oral steroids (addition of azathioprine or 6-mercaptopurine)
Severe	Topical 5-ASA or steroids with increas d dose and duration; topical 5-ASA and steroids; oral steroids	Intravenous steroids (plus intravenous cyclosporin) Surgery
Maintenance	Topical 5-ASA	Oral sulfasalazine or 5-ASA

5-ASA, 5-aminosalicylic acid.

indicate that oral balsalazide is more effective and better tolerated than mesalamine in the treatment of active UC (460). Responses to oral sulfasalazine and oral aminosalicylates are dose related, with up to 80% of patients manifesting clinical remission or improvement within 4 weeks. There is insufficient evidence to confirm a benefit of 5-ASA preparations over sulfasalazine for active or maintenance therapy, but sulfasalazine is not as well tolerated as 5-ASA in active disease despite their relatively similar tolerances in maintenance therapy.

Patients refractory to oral aminosalicylates should be treated with oral prednisone (40 to 60 mg/day) until significant clinical improvement, with a subsequent dose taper of 5 to 10 mg/week until 20 mg and then of 2.5 to 5 mg/week to discontinuation (461). For patients who do not respond to or cannot be weaned from steroids, AZA (1.5 to 2.5 mg/kg/day) has demonstrated effectiveness in achieving and maintaining remission (462,463). Budesonide may also be beneficial for patients with steroid-dependent UC (464).

Severe Ulcerative Colitis

Severe UC is a potentially life-threatening condition, with a mortality, including surgical mortality, that is less than 2% because of the improvements of clinical management. Patients who continue to present with severe symptoms despite optimally dosed oral therapy with steroids and aminosalicylates, as well as topical medications, and patients with evidence of toxicity should be hospitalized and treated with intravenous steroids equivalent to 300 mg of hydrocortisone or 48 mg of methylprednisolone for 7 to 10 days. Patients without prior steroid medication alternatively may profit by intravenous adrenocorticotropic hormone (465–468). Therapy with aminosalicylates should not be initiated during an episode of acute, severe colitis because of possible allergic reactions confusing the clinical picture. Vital signs should be taken repeatedly, and frequent abdominal examination and abdominal radiographs should be performed to detect signs of peritoneal irritation and the presence of small bowel gas and colonic dilation. Patients should receive adequate intravenous fluid and electrolyte replacements. In patients with toxic megacolon, oral nutrition should be stopped, and bowel decompression should be instituted with the passage of a long tube; the patient also should be rolled to a prone position for 15 minutes every 2 to 3 hours to allow redistribution of colonic air. Relief from colonic distention is usually experienced within 24 to 48 hours (469). Adjunctive parenteral nutrition may be useful, but total parenteral nutrition shows no benefit (470). Broad-spectrum antibiotics may be used empirically (471). Surgical consultation should be obtained at the time of admission, because a coordinated effort by gastroenterologists and surgeon can reduce mortality (472). Anticholinergic and narcotic medications have to be avoided in this setting for the fear of worsening colonic atony or dilation.

Up to 35% of patients fail to respond to intravenous steroids (473). Eight or more stools per 24 hours or four to five stools per 24 hours together with C-reactive protein levels above 45 mg/L predict in 85% of patients the need for colectomy after a 3 days therapy with intravenous glucocorticoids (474). Additional management with intravenous cyclosporine in a dose of 4 mg/kg/day has proved effective at reducing the immediate colectomy rate in 80% of patients, but the long-term benefit is much less certain, and one third of patients require surgery within the next 6 months and 60% of patients after 12 months (475–477). Response to intravenous cyclosporine should be seen within 7 days at cyclosporine levels between 300 and 500 ng/mL. Monotherapy with intravenous cyclosporine (4 mg/kg) has also been as effective as 50 mg of an intravenous prednisolone equivalent (478). Major side effects of intravenous cyclosporine were observed in up to 50% of patients, with induction of renal insufficiency being the most frequent (479). Seizures have been associated with low serum magnesium and cholesterol levels because of the cyclosporin hydrophobic vehicle. Administration of intravenous cyclosporine should be performed with careful monitoring of renal function, blood pressure, and magnesium and cholesterol levels. Opportunistic infections, such as *Pneumocystis carinii* pneumonia and cytomegalovirus, have been described (480). Two series demonstrate that 6-MP is beneficial to maintain the initial response to intravenous cyclosporine (481). The efficacy of microemulsion cyclosporine capsules (Neoral) for response maintenance of severe steroid refractory UC remains to be determined. Intravenous and oral tacrolismus may also be successful as an alternative to cyclosporine for patients with severe UC.

Maintenance Therapy in Ulcerative Colitis

Oral sulfasalazine, olsalazine, mesalamine, or balsalazide are all effective as a maintenance regimen in extensive UC by reducing relapse rate fourfold (482–486). Unlike sulfasalazine, use of larger doses of 5-ASA is generally well tolerated. The optimal 5-ASA dose is probably 2 g/day. Although the maximum length of maintenance therapy has not been established, permanent treatment is recommended. In distal UC remission can be maintained by topical therapy with 5-ASA suppositories or enemas (487,488). In patients with remissions not adequately sustained by aminosalicylates, AZA or 6-MP may be useful, but the risk-benefit ratio of indefinite use of these drugs is unknown. In patients with UC encouraging results for methotrexate were reported in open trials and series, whereas a later controlled trial with oral dosing did not show any benefit when compared with placebo (489).

Alternative Treatments for Inflammatory Bowel Disease

In the past few years, new therapeutic concepts for IBD have been formulated based on the increasing insights in the pathophysiology of these diseases aimed at targeting proinflammatory mediators or molecules, aggressive luminal agents, and genetically determined defects. The most significant

issue is the introduction of immunomodulatory treatments using cytokines and anticytokines.

An important role for TNF-α as a pivotal proinflammatory mediator in CD has emerged, which resulted in the development of therapeutic strategies that target TNF-α. Several studies have addressed the potential effect of anti-TNF-α treatment in CD. Administration of the high-affinity human (75%) mouse (25%) chimeric antibody cA2 against TNF-α to patients with treatment-resistent active CD caused clinical response in 65% and remission in 33% of patients (490). The incidences of short-term side effects in the anti-TNF-α–treated group and the placebo group did not differ. Retreatment with cA2 was effective to maintain remission after an initial treatment with cA2 pointing to its benefit for maintenance therapy (491). The same molecule has also shown efficacy for closure of enterocutaneous fistulas (492). The long-term risk profile of cA2 needs to be further evaluated, but especially the potential development of lymphoma seems to be unlikely. The precise mechanism of action is unknown, whether mainly antiinflammatory by neutralizing TNF-α or immune modulatory by altering the function of leukocytes bearing surface TNF-α. The clinical effects of cA2 therapy correlated with downregulation of the production of the T_H1 cytokine IFN-γ by CD2 stimulated lamina propria mononuclear cells, whereas no effect was observed on cytokine production by stimulated peripheral blood mononuclear cells indicating that the primary defect in immune regulation in CD is confined to the mucosal compartments (493). TNF-α antibodies also bind to membrane expressed TNF-α, altering the function of the TNF-α–producing cell and inducing its killing by complement activation (494). Another genetically engineered human antibody to TNF-α, CDP571, has shown effectiveness as a single infusion in patients with active CD (495). Anti-TNF-α seems to be a promising therapeutic strategy in patients who do not respond to standard treatment. The therapeutic efficacy in UC has been controversially described and needs further evaluation (496,497).

IL-10 is a cytokine with antiinflammatory and immunosuppressive properties. Gene-targeted IL-10–deficient mice develop a chronic intestinal inflammatory disease reminiscent of CD. IL-10 administered as a daily bolus injection over 1 week is safe, well tolerated, and may be clinically efficacious in 50% of patients with active steroid-resistent CD (498). The safety of subcutaneous IL-10 treatment was confirmed, but remission was observed in only 29% of treated patients with active CD (499).

The safety and activity of human recombinant IL-11 was evaluated in patients with active CD, and the average percent change from baseline in CDAI score could be significantly reduced (500). An attempt to block leukocyte recruitment to the site of inflammation by ISIS 2302 (ICAM-1 antisense oligonucleotide) in the treatment of steroid-dependent patients with CD may be promising (501). Depleting anti-CD4 antibodies showed only moderate efficacy in CD (502).

The long-lasting local expression and efficacy of antiinflammatory cytokine genes within the intestinal mucosa has been demonstrated by the local transfer of an adenovirus–IL-4 vector in rats with TNBS colitis (503).

Heparin has emerged as a possible therapy for IBD, targeting a potential endothelial dysfunction in regulation of coagulation, inflammation, and vascular repair as an important pathogenetic mechanism in UC (504).

Transdermal nicotine and nicotine enemas induced clinical response in active UC but were paralleled by common side effects (218,505). To improve the safety of nicotine, rectal enema and delayed-release oral nicotine formulations have been developed. To prove the benefit of sucralfate enemas in UC further, larger clinical trials are needed (506).

Despite the concept that luminal bacteria may play a role in the pathogenesis of IBD, antibiotic treatment has been used empirically in IBD for many years. Controlled clinical trials have been generally scarce and studies often lacked adequate statistical power. Data from open studies suggest the efficacy of metronidazole in perianal CD (419,421). A remission-prolonging effect of metronidazole after resection has been suggested (507). Metronidazole has also shown efficacy in controlling active CD, most pronounced in patients with ileocolonic disease (508). Metronidazole may be an alternative in some patients with active CD not responding to sulphasalazine (509). Administration of metronidazole can be complicated by gastrointestinal intolerance and metallic taste, as well as in the long term by peripheral neuropathy, necessitating careful attention to symptoms or signs of paresthesias or neurologic abnormalities. The quinolone antibiotic ciprofloxacin could become an alternative to metronidazole and has been shown efficacy for mild to moderate attacks of CD (510,511). Infection or abscess requires appropriate antibiotic therapy or drainage (percutaneous or surgical).

Adjunctive Therapy in Inflammatory Bowel Disease

Most patients with CD having anemia respond to intravenous iron alone. Erythropoietin has additional effects on hemoglobin concentrations (512). Vitamin D and calcium supplementation prevents bone loss in patients with CD and hormone replacement therapy is beneficial in perimenopausal and postmenopausal women with IBD. Biphosphonates may become alternatives for treatment of metabolic bone disease in IBD (513).

Cancer Surveillance in Inflammatory Bowel Disease

Patients with UC are at increased risk of colorectal cancer in the range of 0.5% to 1% per year after 10 years of extensive disease, and the carcinoma is frequently preceded by dysplasia (514). Dysplasia is characterized by cellular atypia, such as nuclear stratification, loss of nuclear polarity, and nuclear or cellular pleomorphism. Molecular markers such as Ki-67, DPC-4, and DYS may become useful for refining the diag-

nosis of dysplasia or may eventually provide an alternative method for predicting colorectal cancer (515). The presence of low-grade dysplasia is a risk factor with a 20% chance that colon contains cancer, whereas the risk with high-grade dysplasia is up to 40%.

Colitis-associated cancers are more often multiple, broadly infiltrating, anaplastic, uniformly distributed throughout the colon, and they occur in much younger patients than colorectal cancer in the general population. Endoscopic surveillance has been evaluated to detect cancer at an earlier and potential curable stage. In the absence of randomized studies comparing different surveillance protocols, the American College of Gastroenterology recommends an annual surveillance colonoscopy with multiple biopsies at regular intervals of approximately 10 cm in patients with a disease duration of 8 to 10 years (394,516). Uninflamed areas, masses, strictures, and flat lesions should be also biopsied. Chemoprevention of UC-associated colorectal cancer has been suggested by a possible protective effect of folic acid (517).

The risk of colorectal cancer in colonic CD is approximately three times that of unaffected patients, especially in younger patients with long disease duration (518). Surveillance programs therefore may be as appropriate in Crohn's colitis as in UC (519). An increased risk of adenocarcinoma of the small intestine has been observed in CD, notably in patients with young age at diagnosis and patients with extensive small bowel disease (520). However, the overall mortality and lifetime risk due to intestinal cancer seems to be not increased in patients with CD (521).

Surgical Indications and Treatment

Although CD recurs in 50% to 80% of patients within 10 years after resection, surgery often proves to be the swiftest, safest, and most effective route to physical and psychosocial rehabilitation (522,523). For more than two thirds of patients with CD, surgical intervention is indicated. Complications such as obstructing stenoses, suppurative complications, massive hemorrhage, or perforation and intractable disease require surgical intervention. Patients with unresponsive fulminant disease who fail to improve within 7 to 10 days of intensive inpatient management should also be considered surgical candidates. Open laparotomy is the standard procedure for resections in IBD. However, laparoscopic colorectal surgery can be advantageous for treatment of terminal ileal CD, but the definite role of this approach has not clearly emerged (524,525). Strictureplasty has proved an safe and effective treatment for patients with multiple, symptomatic small bowel strictures (526). Nearly 40% of patients with CD will require at least a temporary stoma during their lives, with anorectal disease being the primary indication. Revision surgery for stomal complications is more common after colostomy than ileostomy (527). In selected patients with perianal CD abscess, incision and drainage, fissurectomy, and fistulotomy with Seton drainage are successful, sphincter-sparing techniques. Resection and drainage of intraabdominal abscesses may be preferable to attempted percutaneous drainage and staged resection with anastomosis (528).

The cumulative colectomy rates for UC after 10 years differ between 10% to 30% and depend on the severity of the first attack, the extent of disease at diagnosis and younger age at onset (529,530). Exsanguinating hemorrhage, frank perforation, and documented or strongly suspected carcinoma (high-grade dysplasia or low-grade dysplasia in a mass lesion) are absolute indications for surgery in UC (531–534). Low-grade dysplasia in flat mucosa may also be indicative for surgery, because the 5-year predictive value of such lesions for cancer or high-grade dysplasia is approximately 50%. Patients with severe acute UC unresponsive to intravenous cyclosporine within 7 days require colectomy. Because of the classic dictum of inherent curability by excisional surgery, colectomy should be evaluated at any point for patients with significant deterioration in medical therapy and medically intractable symptoms to regain quality of life (535).

One-, two-, or three-step proctocolectomy with Brooke ileostomy is the standard operation for UC. The procedure is curative and carries the lowest risk of complications. The cosmetically more appealing ileal pouch anal anastomosis (IPAA) preserves the muscularis mucosa of the rectum despite colectomy and maintains bowel continuity. However, the technical failure rate reported approaches 5% to 6%. Acute and chronic pouchitis episodes are seen in up to 50% to 60% and 5% to 10% of patients, respectively (536). Possible etiologic factors for pouchitis include bacterial overgrowth, fecal stasis, mucosal ischemia, intestinal malabsorption, and immune-mediated inflammation (537). The presence of extraintestinal manifestations, PSC, and p-ANCA have been associated with an increased risk of pouchitis, whereas smoking appears to have a protective effect (538,539). Pouchitis is characterized mainly by watery, foul-smelling diarrhea, sometimes containing blood, accompanied by abdominal cramps, urgency, anal soiling, and incontinence. General malaise and low-grade fever may be present. Endoscopically, the mucosal changes can be diffuse or patchy with edematous, hyperemic, granular lesions and punctate ulcers.

The broad-spectrum antibiotics metronidazole or ciprofloxacin are the mainstay of treatment (540,541). Pouchitis intractable to antibiotics requires antiinflammatory or immunosuppressive therapy similar to UC. Even without development of pouchitis, metaplastic, colonic-like changes appear in the pouch mucosa by 6 months after the operation. In long-standing ileoanal pouch severe mucosal atrophy can develop with a risk of neoplastic transformation (542,543). Pouch mucosa should be controlled by endoscopic and histologic surveillance. Generally, IPAA should be avoided in patients with CD because of the risk of disease recurrence within the ileal reservoir and its accompanying debilitating symptoms. However, the controversial notion that IPAA is an acceptable procedure in established CD has been advocated (544–546).

Prognosis

The mortality rate is increased for patients with CD and UC compared with the general population; the increased rate is mainly attributed to IBD-related complications. The principal causes of death in CD are sepsis, perforation, and pulmonary embolism (547,548). Patients with CD have an increased extraintestinal cancer risk of 10% over an unaffected population with the most common tumor type being squamous cell cancer of the skin (549). In UC deaths from colorectal cancer, asthma, and non–alcohol-related liver diseases contribute to an increased mortality rate (550). In studies from southern Europe general mortality was not increased in CD and significantly lower than expected in UC because of a reduced risk of cardiovascular and possibly smoking related deaths (551,552).

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